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EFFECT OF DESIGNATED POLLUTANTS ON PLANTS
Second Annual Report

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TECHNICAL REVIEW AND APPROVAL

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This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

A. A. Thomas

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

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Aluminum oxide particles (Al_2O_3)	Dust generator	Linear regression
Germinating seeds	Ornamental plants	Sodium chloride (NaCl)

20. ABSTRACT (*Continue on reverse side if necessary and identify by block number*)

The phytotoxicity of hydrogen chloride (HCl) gas and aluminum oxide (Al_2O_3) particulates was studied in special plant exposure chambers. Seedlings watered with a salt-enriched (850 ppm NaCl) nutrient solution were more tolerant to damage from HCl than were controls. Seeds germinated after exposure to HCl had reduced seedling lengths compared to controls although germination was not affected. Al_2O_3 alone was not toxic under present test conditions and there was no significant change in plant damage by Al_2O_3 + HCl damage alone. Damage response was more repeatable if humidity was

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held constant. A single short exposure to moderate levels of HCl gas affected a significant loss in final yield if exposure occurred when the plant was a certain age. Plants exposed weekly at sub-phytotoxic concentrations suffered yield reductions in some cases. Mycorrhizal fungi are root symbionts on higher plants. There was no effect on the fungus when mycorrhizal plants were exposed to HCl although ozone-exposed plants experienced reduced mycorrhizae production. A number of plants exposed to HCl were compared using linear regression or probit analysis of the fumigation data. ED_{50}'s estimated that concentration necessary to produce injury on 50% of the total leaves exposed.

PREFACE

This is the second annual report of work performed under the Environmental Toxicology Research sponsored by Air Force Contract F-33615-76C-5005 to the University of California, Irvine. The work under this portion of the contract covers the period from July 1, 1976 to June 30, 1977. This project is titled "The Effect of Designated Pollutants on Plant Species," and was conducted by members of the Statewide Air Pollution Research Center, University of California, Riverside. The study is a continuation of work designed to aid Air Force personnel to recognize and predict the phytotoxic responses of terrestrial plants to air pollutants released by Air Force operations. The study is concerned chiefly with gaseous hydrogen chloride, with an interest in the effect when aluminum oxide particulates are added to the system. The investigations reported here were conducted under greenhouse and laboratory conditions to reduce external variables as much as possible. The plants studied included plants grown commercially or those native to the vicinity of Vandenberg Air Force Base, California.

The cooperation and aid of Air Force contract monitor, Lt. Colonel R. C. Inman, Toxic Hazards Divisions, AMRL, Wright-Patterson Air Force Base, Ohio, has been appreciated. The authors also wish to acknowledge the technical assistance of R. J. Oshima and T. A. Endress of the Air Pollution Research Center for their critical advice; P. McCool for conducting the mycorrhizae study; and A. I. Dickie, M. J. Harris, L. A. Neher, and L. Nolan for their able technical assistance during various parts of the project. The assistance of University of California students S. Kane, D. H. Lick, M. Shulte, and D. A. Small has also been appreciated.

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INTRODUCTION

This project is part of a larger study on the effect on terrestrial and aquatic organisms of potential environmental pollutants released through Air Force operations. The object of this particular phase of the study was to determine the effects of hydrogen chloride (HCl) gas and aluminum oxide (Al_2O_3) particulates on selected plant species. Both of these potential pollutants are formed as by-products when solid rocket fuel burns. The engines developed for the Space Shuttle program release about 55 tons (4.4×10^7 gm HCl, 5.0×10^7 gm Al_2O_3) each of HCl and aluminum oxide (Dawburn and Kinslow, 1976). Although most of this follows the rocket into the upper atmosphere a sizable cloud can remain near the ground (Nadler, 1976). We are concerned with the phytotoxicity of this cloud to vegetation it may contact before dispersing.

Our approach has been limited to short fumigations of representative plant species. Plants were exposed for 5 to 20 minutes in special chambers. Pollutants have been limited to HCl gas alone or with Al_2O_3. Aluminum oxide alone has also been tested but does not seem to be phytotoxic. We are also concerned with the effect of HCl on seeds and absorption of the acid by plants through the soil.

Considerable time has been spent designing the delivery systems and exposure chambers capable of producing and containing known amounts of HCl and Al_2O_3. Previous work (Granett and Taylor, 1976; Lerman, 1976; Lerman et al., 1976) have described some of this equipment. The chambers are dynamic, allowing about 2 changes of filtered greenhouse air per minute. HCl gas is added to this air by vaporizing acid solution or by introducing pressurized dry gas. Aluminum oxide is added by using a special dust generator described in an earlier report (Granett and Taylor, 1976).

Detection of HCl gas in the chamber during fumigation is by either wet-chemistry analysis of a sample scrubbed from the chamber atmosphere or by use of the Geomet HCl monitor, a chemiluminescent device. Much time was spent calibrating the Geomet instrument.

Plants exposed to pollutants were graded for visible damage symptoms. HCl usually produces necrotic burning of leaf tissue. Considerable plant tissue must be killed to effect death of the entire plant. The interaction of environmental factors such as light, temperature, and humidity are of interest to the pattern of plant sensitivity. Longer studies are designed to test the effect of one or more short exposures on the ultimate growth and yield of the exposed plant.

This annual report details the continuing construction and calibration of equipment, various tests for determining HCl concentrations for certain damage levels, and other studies on the interaction of pollutants and biological systems.

MATERIALS AND METHODS

EXPOSURE EQUIPMENT

Lexan chamber

A rectilinear chamber measuring 0.86 m high, 1 m wide and 0.75 m deep has been constructed of 1/4 inch Lexan plastic. Figures 1 and 2 show the chamber and auxiliary equipment. Charcoal filtered greenhouse air is delivered to the chamber through a high velocity fan, a preconditioning chamber, and a 1 m long plexiglas tube 4 inches in diameter. Air exhausts through 140 holes, 1/4 inch diameter, in the epoxy painted wooden base to a 2 inch diameter PVC tube connnected to an outside chimney through an exhaust fan. The high velocity and exhaust fans were adjusted to provide about 1/4 inch negative static water pressure with an air movement of 30 cfm. There are 2 changes of air per minute. A motor drives mixing blades in the chamber ceiling at 120 rpm. At the further end of the 4-inch plexiglas tube, gaseous or particulate pollutants are introduced by specific generators. Gas is generated for this chamber by volatilized HCl solutions (Granett and Taylor, 1976).

Aluminum oxide is supplied to the Lexan chamber using the dust generator described by Granett and Taylor (1976). A special HCl-Al_2O_3 mixing device has been designed and tested. Illustrated in Figure 1B, it is more fully described later in this report.

The Lexan chamber has a pre-chamber, 0.4 m high, 0.6 m wide and 0.6 m deep, where the humidity of the air is increased before it enters the main chamber. First attempts to increase the water content of the air were not successful; water saturated cheesecloth panels elevated the relative humidity only 1-5%. Likewise, only small increases were noted when cold or hot water was atmoized into the pre-chamber. For one experiment, water was atomized into the main chamber directly and the humidity rose to 100%, but the resulting mist was undesirable. To provide a constant and controllable source of humidity, live steam is introduced into the pre-chamber. A single steam valve controls the amount added to increase chamber humidity to above 75%.

Cylindrical chambers

Two additional chambers have been built using plans provided by Dr. H. Rogers at North Carolina State University (Rogers, 1975; Jeffries et al., 1976). See Figures 3 and 4. These cylindrical chambers are constructed of welded steel bars covered with 2 mil Teflon film. The chambers are 1.1 m in diameter and 1.2 m high. Effective gas mixing is achieved with blades revolving at 120 rpm and by 3 vertical baffles 10.5 cm inches wide mounted equidistant around the chamber perimeter. The base forms a plenum with 115 holes, 5/16 inch diameter. Filtered greenhouse air is drawn through a 1.2 m long 3-1/2 inch diameter PVC intake manifold and enters the top of the chamber. The air continues through the plenum and exhausts through a 2 inch PVC pipe. The exhaust manifolds join at a junction box where air flow

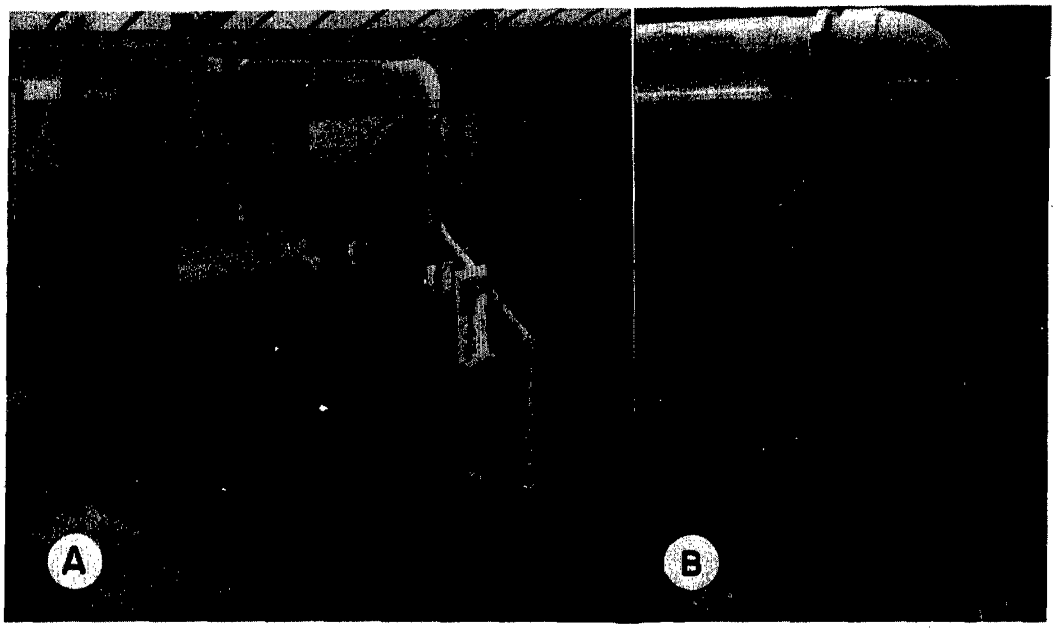

Figure 1. Photographs of Lexan fumigation chamber. 1A, Chamber with dust generator, pre-chamber, and sampling equipment, 1B, Detail of dust generator exhaust, glass mixing device, intake manifold, and HCl vapor lines.

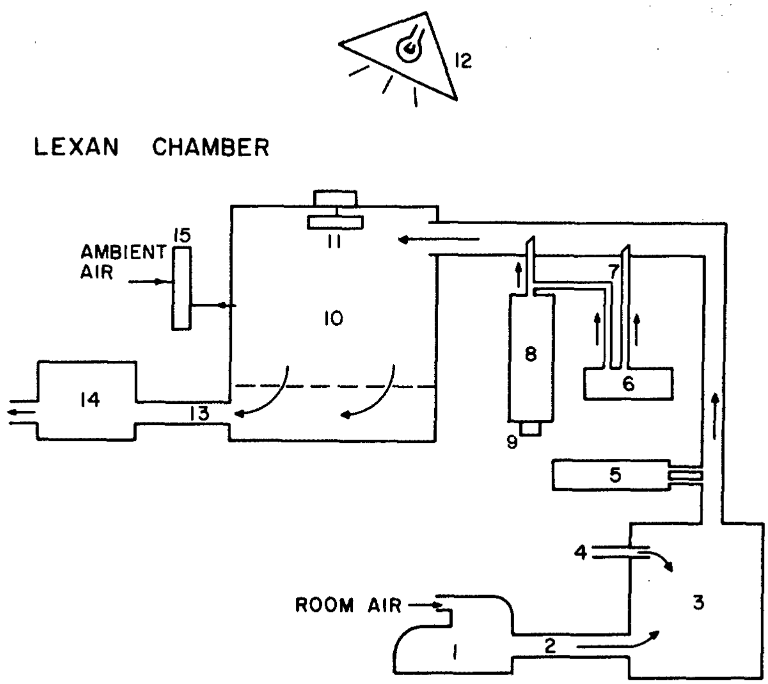

Figure 2. Diagram of Lexan chamber. KEY: 1, Input blower; 2, Intake manifold; 3, Pre-chamber; 4, Steam line; 5, Restricted orifice flowmeter; 6, HCl gas generator; 7, HCl vapor lines; 8, Dust generator; 9, Dust reservoir; 10, Exposure chamber; 11, Mixing blades; 12, Halide lamp; 13, Exhaust manifold; 14, Exhaust fan; 15, Pressure differential gauge.

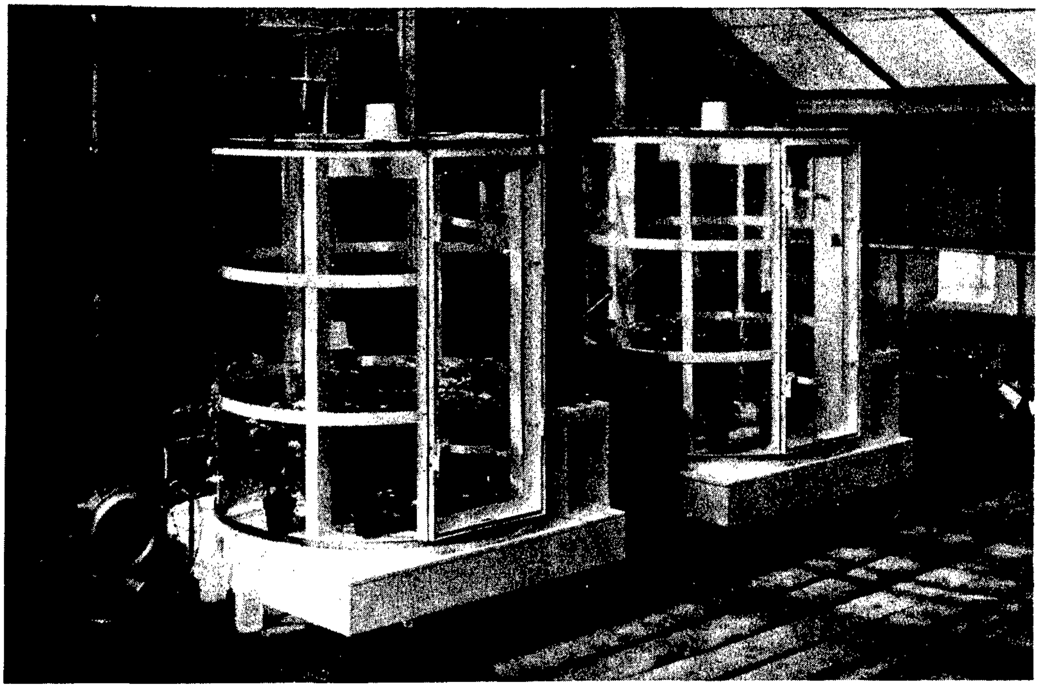

Figure 3. Photograph of cylindrical chambers and auxiliary equipment.

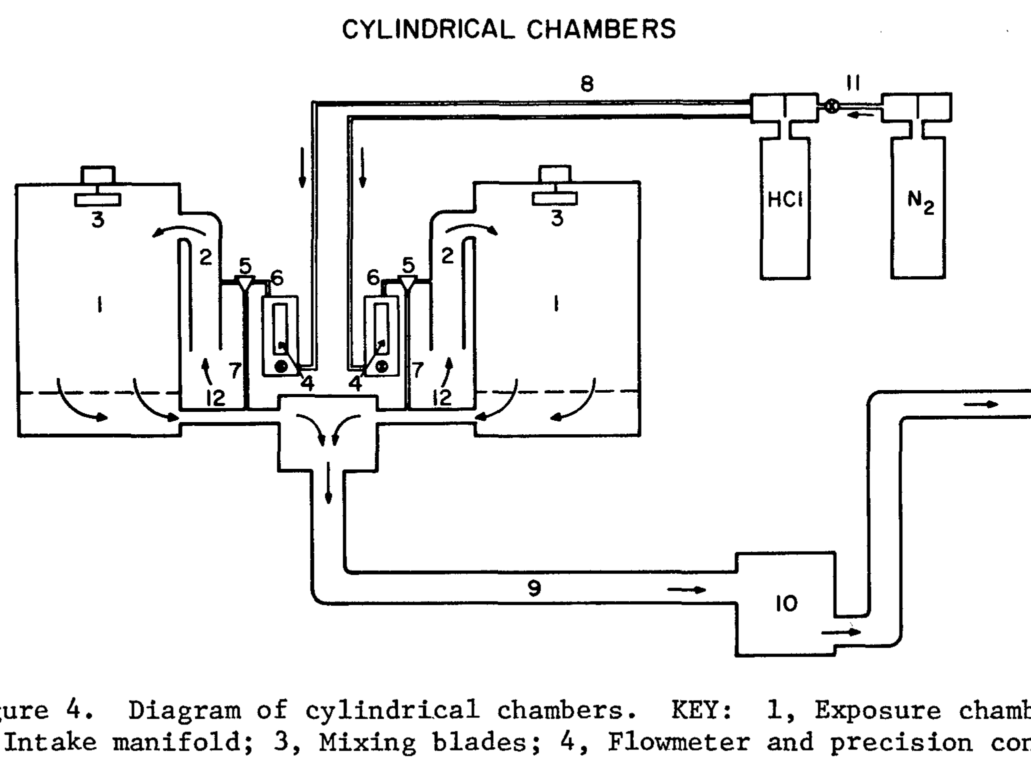

Figure 4. Diagram of cylindrical chambers. KEY: 1, Exposure chamber; 2, Intake manifold; 3, Mixing blades; 4, Flowmeter and precision control valve; 5, Shunt valve; 6, Chamber input line; 7, Bypass line; 8, HCl supply line; 9, Exhaust manifold; 10, Exhaust fan; 11, Nitrogen purge system; 12, Filtered greenhouse air.

can be controlled with a diaphragm. Four-inch-diameter PVC pipe connects a single squirrel cage exhaust fan with the junction box and an outside chimney. Air movement is 1300 cfm or nearly two changes a minute. There is no variation in air flow in one chamber when the other is opened.

A dry gas delivery system supplies the pollutant to the cylindrical chambers and is diagrammed in Figure 5. Forty percent HCl pressurized with dry nitrogen gas is supplied to two Matheson #602 flowmeters, one for each chamber. A 2-way control valve can direct the gas to the chamber or by-pass it to the exhaust manifold. When the valve is set in the center, no gas flows past it. A 15-turn needle valve provides precision control of the gas flow. All connection lines are 1/8-inch-diameter Teflon. To minimize corrosion, especially in the valves and flowmeter, dry nitrogen gas purges the entire system after HCl use.

POLLUTANT MEASUREMENT

Measurement of HCl

Hydrogen chloride gas concentration in the chambers is measured by an air-scrubbing impinger. This system can be seen in Figure 2 and is diagrammed in Figure 6. A measured amount of chamber gas, usually 15 liters, is drawn through a unit containing 20 ml of 0.1 N HNO_3. The solution is removed and analyzed for chlorine with an automatic titrator and the amount of HCl in the chamber is calculated in mg HCl m^{-3}. For sea level elevation at Riverside, California, 1 ppm is equal to 1.52 mg HCl m^{-3}.

Also available for measuring chamber concentration is a Geomet HCl monitor (Model 401S). This chemiluminescent device registers concentrations directly in mg m^{-3}. Although very useful for observing rapid changes in gas concentration, the instrument has been extremely difficult to calibrate so its values correspond to the impinger measurements. The Geomet monitor is presently used as a secondary check since the disagreement ranges from 0 to 25% higher or lower in a single experiment.

Measurement of Al_2O_3

Aluminum oxide was not measured directly for the current experiments. The generator is adjusted before each exposure for desired concentration according to a calibration chart. Previous work (Granett and Taylor, 1976) indicated close correlation between expected and measured concentrations.

CHAMBER CALIBRATION

Lexan chamber

The Lexan chamber was calibrated and tested in several ways. Comparisons

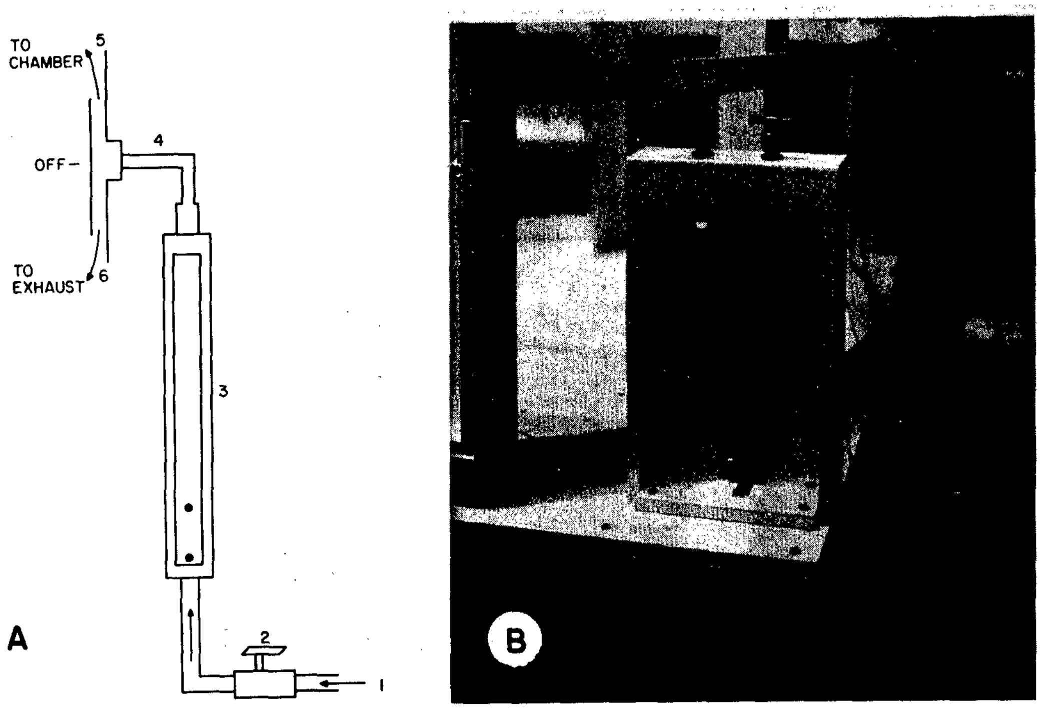

Figure 5. Dry HCl gas control system. 5A, Diagram of system, KEY: 1, 40% HCl supply line; 2, Precision needle valve; 3, Flowmeter; 4, Shunt valve; 5, Teflon line to intake manifold; 6, Teflon line to exhaust manifold; 5B Photograph of system.

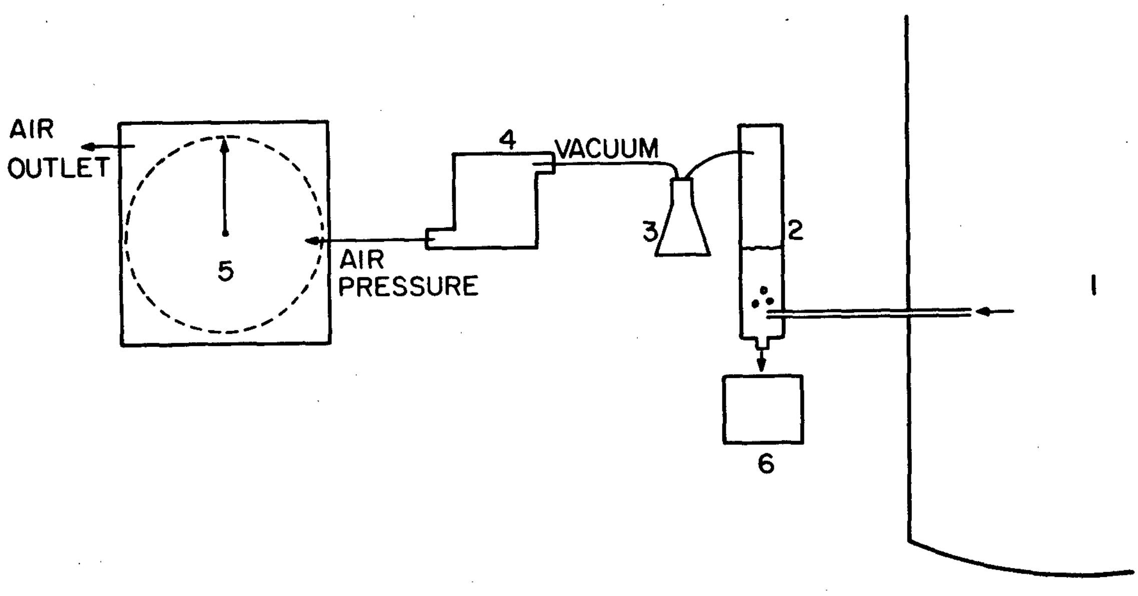

Figure 6. Diagram of chamber atmosphere sampling system. KEY: 1, Chamber atmosphere; 2, Impinger with 0.01 N nitric acid; 3, Fluid trap; 4, Air pump with vacuum and pressure ports; 5, Wet test meter; 6, Sample for titration.

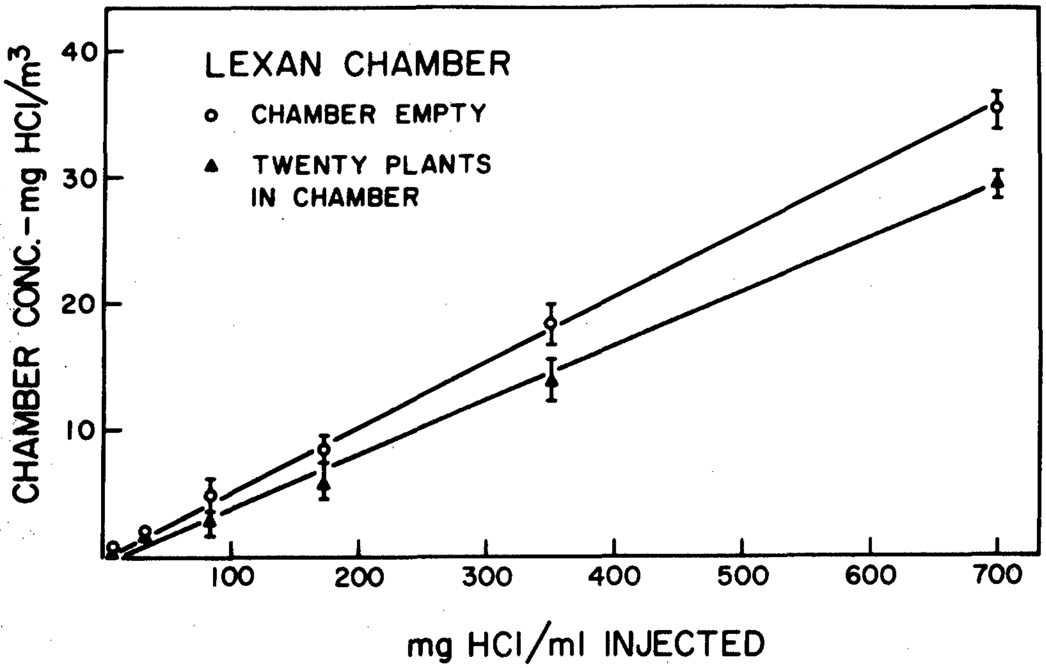

Figure 7. Lexan chamber calibration curves with and without plants in the chamber

were made with the older Teflon-covered, wooden-framed, rectilinear chambers: plant reaction was the same and gas mixing was the same or better. Daylight was measured in the two chambers and light intensity (ergs cm^{-2} sec^{-1}) was the same in both. Light in the photosynthetic region (microeinsteins cm^{-2} sec^{-1} at 400-700 mm) passed more easily through Teflon than Lexan with 6% and 17% losses, respectively. Calibration curves (Figure 7) were developed by calculating the amount of HCl gas delivered and graphing it against measured chamber concentrations under empty and loaded conditions.

<u>Cylindrical chamber</u>

The cylindrical chambers were tested for efficiency in gas mixing by supplying 25 pphm ozone into the intake manifold. The ozone concentration was then measured with a Dasibi Corporation (Model 1003) ozone monitor at three chamber levels and at five positions at each level. The minor daily fluctuations were probably due to temperature and light fluctuation rather than insufficient mixing. Table 1 shows the data for the experiment. Using a two-way analysis of variance, there was no significant difference between any value.

The cylindrical chambers were also calibrated for HCl. The flowmeters were set at calculated flows and resulting chamber concentrations were measured using the impinger and Geomet. The chambers were so tested with and without plants to construct working calibration curves relating flowmeter setting with chamber concentration. The two lines are presented in Figure 8 and allow setting of the chamber concentration using the flowmeter

Table 1. Distribution of ozone in cylindrical chamber.

Position[1]	Height above base (cm) 15	50	80	Avg
1	25 ± 3[2]	25 ± 4	27 ± 5	26
2	27 ± 2	25 ± 3	27 ± 1	26
3	26 ± 2	26 ± 3	25 ± 4	26
4	26 ± 3	29 ± 3	24 ± 3	26
5	26 ± 2	25 ± 3	25 ± 2	25
Avg	26	26	26	

[1]Position on horizontal plane in quartered chamber. Position 5 is chamber center.

[2]Mean and standard deviation of 2 to 5 readings at each position in chamber, in pphm ozone. ANOVA determined that there was no significant difference between values for height, for position, or for the interaction of the two. Corrected Bartlett's test also showed non-significance.

now in the system. By calculating HCl gas delivered to the chambers, another set of calibration curves could be drawn which was not dependent on flowmeter type. These curves are presented in Figure 9. The two chambers correspond closely enough to use the calibration curves interchangeably. Accuracy of obtaining desired chamber concentration is about 10 to 15%, being influenced by temperature, light, and number of plants in the chamber.

Plants and pots as HCl sink

We investigated how much HCl plants and pots adsorb and whether this adsorption significantly lowers chamber HCl concentration during a short fumigation. Vaporized HCl was supplied to the Lexan chamber at a constant 23 mg HCl m^{-3}. The concentration was measured by scrubbing an air sample in the usual manner before plants were introduced and again 5 minutes after they had been in the chamber. Four, 8, 12, or 20 zinnia plants, 30 days old and in four inch pots were tested. Controls, introduced separately, consisted of 4, 8, 12 or 20 four inch pots containing moist soil but no plants.

The change in chamber concentration was calculated and a measure of plant size was obtained with a Lambda Instrument Corporation leaf area meter (Model LI-3000) with belt conveyer assembly (Model LI-3050A). The data are summarized in Table 2 and Figure 10. The chamber concentration generally decreased as more surface area was introduced. Pots and soil

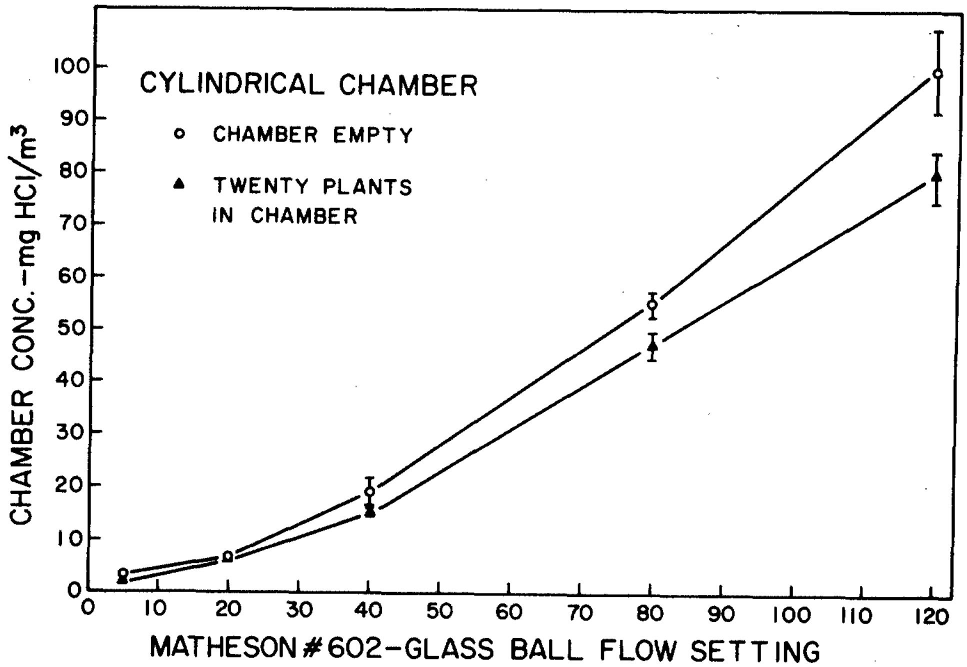

Figure 8. Working calibration curves for cylindrical chambers: amount of gas through Matheson #602 flowmeter produces certain chamber concentration.

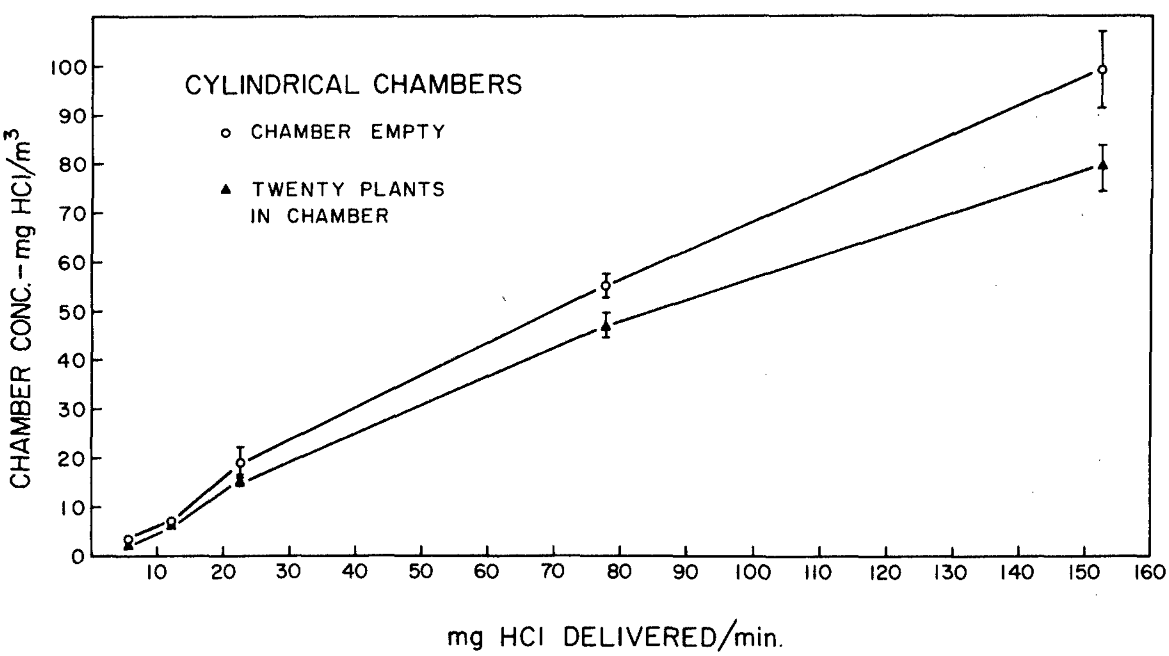

Figure 9. Cylindrical chamber calibration curves based on calculated HCl dry gas delivered.

Table 2. Change in chamber HCl concentration as related to chamber load.

Zinnia plants			Pots without plants	
Leaf area[1]	Number plants	% change in HCl chamber concentration	No. pots with soil only	% change in HCl chamber concentration
278[1]	4	- 3.9[2]	4	- 8.5[2]
405	4	-14.3	4	3.0
549	4	2.7	4	- 6.8
640	8	-17.8	8	0
1002	12	-18.2	8	- 5.4
1120	8	-10.6	12	-10.0
1144	12	-13.5	12	-15.5
1661	20	-17.6	12	- 5.4
1691	12	-21.1	20	3.0
2149	20	-16.6	20	14.9
3096	20	-28.7	20	15.9

[1]Area is total surface area, one side, in cm^2

[2]A negative percent indicates lowering of HCl concentration from approximately 23 mg m^{-3}

are responsible for an important part of the reactive surface area. For the short fumigations under investigation, these changes in chamber concentration must be taken into account.

OPERATION SAFETY

Since HCl is a toxic substance all precautions are taken in its use. Concentrated solutions are kept under hoods. The Teflon lines in the pressurized dry gas system are secured with Swagelok fittings and are frequently checked for leaks with "Snooper" solution. All exposure chambers operate with negative pressure so that greenhouse air goes into the chamber when it is opened or if leaks develop. Exposure chamber air is exhausted through a four inch diameter PVC tube 4 m above the ground. The Geomet HCl monitor was used to measure gas concentration around the chamber and exhaust outlet.

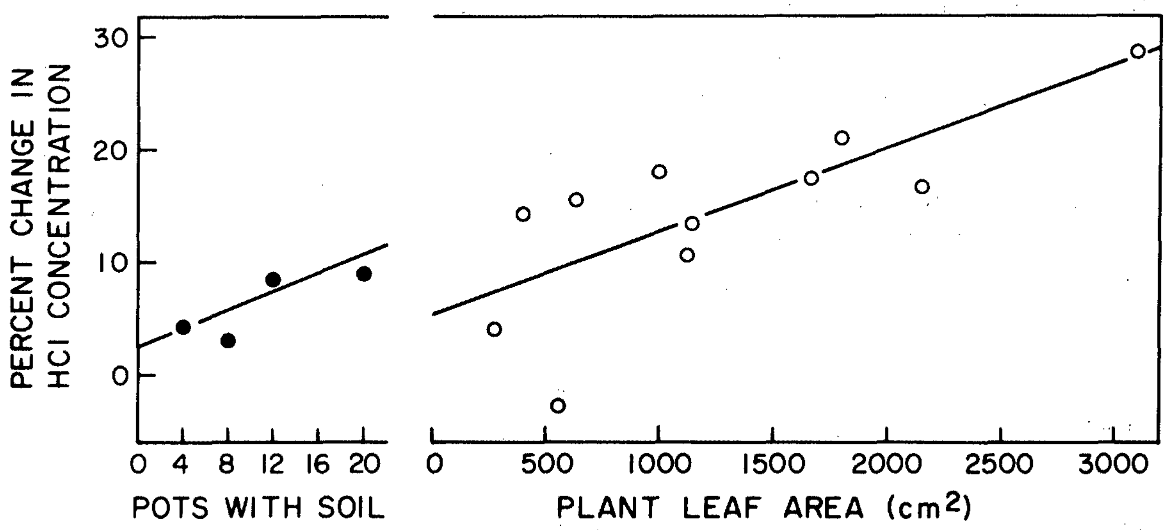

Figure 10. Change in HCl concentration in exposure chamber with increasing plant surface area and increasing numbers of soil-filled pots.

Table 3 shows the HCl concentrations detected in various locations. The HCl gas decreases rapidly as it travels from the chamber. From this work we conclude that the toxic gases generated for our studies are readily dispersed and pose no environmental or personnel hazard.

Table 3. Detection of HCl gas around chamber and exhaust tube.

Location	HCl Detected (mg HCl m^{-3})
Greenhouse	
In chamber	30
Outside chamber door & other parts of chamber	0
Outside	
In exhaust tube outlet	15
0.5 m downwind from outlet	3
0.5 m upwind from outlet	0.2
0.5 m below outlet	0.2
1 m from exhaust outlet, any direction	< 0.1

PLANT PRODUCTION

Greenhouse conditions

The plants used for these studies were usually grown from seed in Greenhouse 21 of the Air Pollution Research Center, University of California, Riverside. This building is kept pollution- and pest-free by a charcoal filtered air system and a rigorous pest management program. UC soil mix II, previously described by Lerman (1976, 1977) is sterilized. A complete nutrient solution described by Hoagland and Arnon (1950) is given to each plant one to several times a week. Plants are watered as needed with deionized water. Daily greenhouse temperature maxima were between 34 and 40 C while night temperatures were between 18 and 23 C. Temperatures were maintained by evaporative coolers, glass white-washing, and steam heat. Other soil, temperature regimes, or growing conditions are described more fully for specific experiments.

Plants

Table 4 lists the plants under study during the period covered by this report. New plants were chosen for certain reasons; briza, coreopsis, and wallflower are closely related to species native to Vandenberg Air Force Base. Avocado, calendula, citrus, and petunia are likely to be found commercially in the Lompoc area. A source of Bishop pine seeds enabled us to begin experiments with very young, uniform plants.

Plant exposure

Plants were usually watered prior to introduction into the exposure chamber. During exposures at high gas concentrations plant stress was noted. Continued stress, which appeared to be a wilted condition at first, developed into a collapse of interveinal regions shortly after exposure was completed. Plants were removed to the greenhouse bench after fumigation where they often recovered from stress conditions. Injury, which occurred on leaves 6 to 24 hours post-exposure, was manifested as abaxial glazing or necrosis depending on severity of exposure. It was very rare for a plant to die after exposure to HCl gas at the doses used.

PHYTOTOXICITY TESTS

SALT NUTRITION AND THE SENSITIVITY OF PLANTS TO HCl GAS

Plants growing in coastal environments are exposed to increased levels of chlorine in the form of salt sprays and aerosols. Increases in plant chloride content can sometimes be found as far as 50 miles inland (Ogden, 1975). Plants with elevated chlorine levels may be more sensitive to exposure to HCl gas.

Table 4. List of plant species and varieties used in phytoxicity studies.

Plant	Scientific name	Variety
Aster	*Callistephus chinensis* (L.) Nees	Early bird white
Barley	*Hordeum vulgare* L.	CM 67
Bean	*Phaseolus vulgaris* L.	Pinto, U.I.III
Briza	*Briza maxima* L.	Ornamental quaking grass
Calendula	*Calendula officinalis* L.	Flame beauty
Citrus	*Poncirus trifoliata* (L.) Raf. X *Citrus sinensis* (L.) Osbeck	Troyer citrange
Coreopsis	*Coreopsis grandiflora* Nutt.	Sunburst
Marigold	*Tagetes patula* L.	French dwarf double goldie
Marigold	*Tagetes erecta* L.	Senator Dirksen
Petunia	*Petunia hybrida* Vilm.	White cascade
Pine	*Pinus muricata* D. Don	Bishop pine
Radish	*Raphanus sativus* L.	Comet
Sugar beet	*Beta vulgaris* L.	U.S. H-10
Tomato	*Lycopersicon esculentum* Mill	Ace
Tomato	*Lycopersicon esculentum* var *cerasiforme* (Dun.) A. Gray	Tiny tim
Wallflower	*Cheiranthus allioni* L.	Golden bedder
Zinnia	*Zinnia elegans* Jacq.	White gem

Materials and Methods

To test whether this was true, pinto bean and zinnia seedlings were grown in a soil watered with the normal nutrient solution plus 0 or 850 ppm NaCl. Two weeks after supplemental watering began, the plants were exposed to HCl gas at 10 or 30 mg m^{-3}. Twenty-four hours after exposure the plants

were graded for injury, leaves were removed, and fresh weights recorded. Leaf areas were measured with a Lambda area meter. The leaves were oven-dried at 70 C, re-weighed, powdered, and eluted in weak acid in order to measure total chlorine using the automatic titrator.

Results and Discussion

Table 5 summarizes the data from the experiment. Student t-tests were used to compare the total leaf areas, leaf fresh weight, and injured areas. As noted, there was no significance at the 5% level for the zinnia seedlings. The pinto beans, however, showed significant differences with plants receiving salt-enriched nutrients being more resistant to damage by HCl gas than plants without excess NaCl. The salt seemed to retard bean leaf expansion for the plants had a smaller leaf area and weighed less than plants grown under more normal conditions.

Table 5. Effect of short exposures of HCl gas on pinto bean and zinnia seedlings grown with NaCl-enriched nutrient solution.

	Pinto bean			Zinnia		
	No NaCl		+ NaCl	No NaCl		+ NaCl
Number of plants	24		24	23		25
Cl^- supplied to plant (mg)	0.1		146	0.1		133
Number leaves injured	19		9	24		19
Total leaf area/plant (cm^2)	99	(*)	88	38	(NS)	40
Weight of leaves/plant (mg)	2050	(NS)	2030	760	(NS)	830
Total leaf area injured/plant	19%	(*)	7%	21%	(NS)	12%

*pair is significantly different at 5% level by student t-test

NS shows no significant difference by t-test

Table 6 breaks down the injury data into concentration and time subgroups. There was negligible injury on the plants at low HCl concentrations. At the higher concentrations, injury was greater when plants were not stressed with salt. This was more evident with the pinto bean than with the zinnia data. As the exposure period increased, the plants which had received no NaCl seemed to show more injury than the NaCl-treated plants.

Table 7 lists the chloride content in the leaf tissue for the various treatments. As expected, there was more chloride in those plants which had

Table 6. Injury of pinto bean and zinnia seedlings supplied with nutrient solutions supplemented with NaCl before exposure to HCl gas.

Exposure duration (minutes)	Pinto bean		Zinnia	
	No NaCl	+ NaCl	No NaCl	+ NaCl
Control				
0	0[1]	0	0	0
Low Concentration HCl ($10\ mg\ m^{-3}$)				
5	1	0	1	0
10	0	1	0	0
15	0	0	0	0
20	1	0	0	0
High Concentration HCl ($30\ mg\ m^{-3}$)				
5	15	2	1	1
10	34	4	37	11
20	26	22	19	19

[1]Injury expressed as severity-weighted %-leaf area injured per plant, average of 3 plants

Table 7. Chloride content of leaves from pintc bean and zinnia seedlings supplied with NaCl before exposure to HCl gas.

Exposure duration (minutes)	Pinto bean		Zinnia	
	No NaCl	+ NaCl	No NaCl	+ NaCl
Control				
0	0.70	2.52	0.68	1.39
Low Concentration HCl ($10\ mg\ m^{-3}$)				
5	1.52	1.99	0.70	1.33
10	--	--	0.49	1.61
15	1.71	1.89	0.95	1.84
20	1.35	2.61	0.83	1.19
High Concentration HCl ($30\ mg\ m^{-3}$)				
5	0.83	3.33	0.66	1.60
10	1.65	3.45	1.34	1.50
20	1.66	1.56	1.01	1.84

[1]Chloride content expressed as %-Cl^- per mg dry leaf tissue

received supplemental NaCl. Although more was found in the bean than in the zinnia leaf tissue, generalizations on the effect gas fumigations had on the chloride levels is difficult due, in large part, to data variability.

Table 8 is a summary of the three-way analysis of variance (ANOVA) for the data. Large significant differences were found between fumigation treatments (HCl exposures) with respect to leaf injury (both percent leaves and area) but not to leaf chloride content. Differences in all variables, injury and chloride content, were noted when comparing NaCl treatments. There were significant differences seen between bean and zinnia seedlings (species) with respect to percent leaves injured and leaf chloride content. Only the HCl x NaCl interaction was significant and that only in terms of leaf area. This supports the findings recorded in Table 6 that the NaCl-treated plants responded differently in response to the HCl gas.

When Thomas (1976) grew nasturtiums under hydroponic conditions, he found that five times normal chlorine or 90 ppm in the circulating nutrient resulted in greater damage from subsequent exposure to HCl gas. The present results indicate no increased sensitivity to gas damage with 500 ppm chlorine (850 ppm NaCl) applied to the soil. Bean plants seemed to show some resistance to damage but their leaves were smaller.

EFFECTS OF HCl ON SEEDS

Newly sown seeds or seeds drying in a mature flower head may be sensitive to HCl gas. In a previous report Lerman (1976) reported on seed germination studies involving solutions of HCl, hydrogen fluoride (HF) and mixtures with Al_2O_3. He found root length inhibited almost 75% by 0.02% HCl. Developing seeds may react differently to gaseous toxicants than to solutions. With this in mind, several tests were made .

Materials and Methods

In Lerman's work (1976), seeds were surface sterilized by rinsing for several minutes with sodium hypochlorite. This practice may have left some chlorine residue or might have otherwise predisposed the seeds to increased injury from HCl gas. A preliminary study revealed that the disinfectant was not necessary with either CM67 barley or Ace tomato seeds. No disinfectant rinse was used, therefore, in the following seed experiments.

Both the barley and tomato seeds were allowed to imbibe water prior to exposure. One group of each species was given additional time to germinate prior to exposure by transferring the imbibed seeds to petri plates with moist filter paper and keeping the closed plates in the dark. Other groups were fumigated immediately after imbibition. Initiation of imbibition was staggered so that all seeds of the same species could be fumigated at the same time. Seeds were all transferred to petri plates with dry filter paper before exposure to gas.

Barley seeds were allowed 18 hours imbibition. The pre-exposure

Table 8. Analysis of variance summary for NaCl-supplement experiment.

Variables	Degrees of freedom	F[1]
Percent leaves injured		
HCl exposure (HCl)[2]	7	18.98***
NaCl treatment (0 or 850 ppm NaCl)	1	6.66*
Species	1	10.11*
HCl x NaCl interaction	7	1.54
HCl x Species interaction	7	1.53
NaCl x Species interaction	1	1.89
Experimental error	7	-
Sampling error	64	-
Total	95	
Percent leaf area injured		
HCl exposure (HCl)	7	46.41***
NaCl treatment (NaCl)	1	19.45**
Species	1	2.33
HCl x NaCl interaction	7	7.61**
HCl x Species interaction	7	1.79
NaCl x Species interaction	1	2.37
Experimental error	7	
Sampling error	64	
Total	95	
Leaf tissue chloride content		
HCl exposures (HCl)[3]	6	0.64
NaCl treatment	1	19.26**
Species	1	12.20*
HCl x NaCl interaction	6	0.79
HCl x Species interaction	6	0.40
NaCl x Species interaction	1	1.44
Sampling error	6	-
Total	27	

[1]Statistical F-value with * = 5%, ** = 1%, and *** = 0.1% levels of significance

[2]HCl exposure treatments are 0, 10, and 30 mg HCl m^{-3} for 5,10,15, and 20 minutes

[3]Chloride data not available for plants at 10 mg m^{-3} for 10 minutes

germination consisted of 0 or 24 hours in the dark. Ten seeds were exposed in each of three open petri plates and the exposures consisted of concentrations of either 0 or 25-30 mg HCl m^{-3} for 0, 5, or 20 minutes. The entire series was replicated on the same day. After exposure, the filter paper was moistened and all petri plates were covered and returned to a growth chamber for germination at 22 C in the dark. The length of the epicotyl and the radicle of germinated seeds was measured 48 hours after imbibition. With tomato seeds, the imbibition soak lasted 24 hours and the pre-exposure germination period was 0 or 48 hours. After the 48-hour period, only seeds showing signs of growth were selected for fumigation. Each pretreatment was represented by two petri plates or 50 seeds. The whole series was replicated in a single day. All exposures were 20 minutes long with HCl concentrations averaging 0, 21, or 38 mg m^{-3}. After exposure the petri plates were covered and placed in the dark growth chamber. Germinated seeds were measured for total hypocotyl plus radicle length 168 hrs (7 days) after imbibition.

Results and Discussion

Tables 9 and 10 are the summary and ANOVA for the barley seed data. Seed germination was quite good and the small reductions with different treatments are not highly significant. Seedling length, however, exhibited differences between HCl concentrations, exposure times and the pre-exposure germination period. There was no significant difference between replicas.

Table 9. Effect of HCl gas on the germination and early development of CM-67 barley seeds.

	Pre-exposure treatments[3]			
	0 hours		24 hours	
Exposure[1] period	Germination[4] percent	Average[5] length (mm)	Germination[4] percent	Average[5] length (mm)
Control[2]	95	85	99	122
5 minutes	98	92	98	96
20 minutes	82	59	90	64

[1]Exposures were at 25-30 mg HCl m^{-3} except the controls

[2]Control seeds were subjected to 0, 5, or 20 minute exposures of filtered air

[3]Pre-exposure germination was the period between imbibition and fumigation

[4]Average germination of 180 or 60 seeds for controls and gas exposures, respectively

[5]Average length of epicotyl plus radicle for 180 or 60 seeds

Table 10. Analysis of variance summary for barley seeds exposed to HCl gas.

Source of Variation	Percent germination DF[2]	Percent germination F[3]	Total length DF[2]	Total length F[3]
HCl concentration (HCl)	1	10.29*	1	133.48***
Exposure time (Time)	1	18.29*	1	333.70***
HCl x Time interaction	1	14.00*	1	24.26**
HCl x 5 minute	1	0.14	1	21.97**
HCl x 20 minutes	1	24.14**	1	135.77***
Experimental error 1 (HCl, Time)	4	-	4	-
Pre-exposure germination (Germ.)	1	0.77	1	15.23*
HCl x Germ. interaction	1	0.00	1	5.52
Germ. x 0 mg m^{-3}	1	0.38	1	19.54*
Germ. x 30 mg m^{-3}	1	0.38	1	1.21
Time x Germ. interaction	1	0.49	1	0.23
HCl x Time x Germ. interaction	1	0.03	1	2.38
Experimental error 2	4	-	4	-
Sampling error	32	-	464	-
Total	47	-	479	-

[1]Pre-exposure germination periods were 0 and 24 hours

[2]DF = degrees of freedom

[3]F-values with significance at * = 5%, ** = 1%, and *** = 0.1%

For the tomato seeds the data are reviewed in Tables 11 and 12. That germination seems lower with no pre-exposure germination period (0 hr) is misleading since seeds that had not begun to germinate were not selected for the subsequent fumigations. All seeds exposed to gas showed differences in seedling lengths attributable to the gas concentration and the pre-exposure period.

In summary, there was significant reduction in both barley and tomato seedling lengths when the seeds were exposed to moderately phytotoxic levels of HCl. Germination does not seem to be greatly reduced by the pollutant, nor do the seeds seem to be much more sensitive if exposed when partially germinated. Although seedling length may be reduced by HCl, there is preliminary evidence (Granett, unpublished data) that soil may act as a buffer to reduce or eliminate the harmful effects of the gas.

Table 11. Effect of HCl gas on the germination and development of Ace tomato seeds.

HCl gas concentration (mg m^{-3})	Pre-exposure treatments[2] 0 hours Germination percent	0 hours Avg. length (mm)	48 hours Germination percent	48 hours Avg. length (mm)
0	88[3]	73.0[4]	100[3]	64.6[4]
21	80	6.4	100	9.9
38	74	1.5	100	4.6

[1]Average of two 20-minute exposures

[2]Pre-exposure treatment was the period of time between imbibition and fumigation

[3]Percent of 100 seeds that germinated

[4]Average length of hypocotyl plus radicle

Table 12. Analysis of variance summary for tomato seeds exposed to HCl gas.

Source of Variation	Percent germination DF	Percent germination F	Total length DF	Total length F
HCl concentration (HCl)	2	1.34	2	1084.22***
Pre-exposure germination (Germ.)	1	30.81**	1	11.34*
HCl x Germ. interaction	2	1.34	1	25.36**
Germ. x 0 mg m^{-3} interaction	1	3.82	1	60.06***
Germ. x 21 mg m^{-3} interaction	1	11.71*	1	0.60
Germ. x 38 mg m^{-3} interaction	1	17.95**	1	1.34
Experimental error	6	-	6	-
Sampling error	12	-	588	-
Total	23	-	599	-

[1]Pre-exposure germination periods were 0 and 48 hours

[2]DF = degrees of freedom

[3]F-values with significance at * = 5%, ** = 1%, and *** = 0.1%

ALUMINUM OXIDE AND PLANT INJURY

Tests to determine whether HCl gas plus aluminum oxide (Al_2O_3) particles produce more plant injury than HCl gas alone under the same conditions have continued. Earlier work (Lerman, 1976; Granett and Taylor, 1976) had indicated that there was no injury from aluminum oxide alone and an insignificant increase when the two toxicants were combined. The results of the current tests further confirm this.

Effect of Al_2O_3 and HCl on zinnia

Materials and Methods

In one experiment zinnia plants were exposed for 20 minutes to HCl or HCl plus Al_2O_3 when they were 27 days old. Eight plants were exposed during each fumigation. The Al_2O_3 was in the sub-micron range as previously characterized (Lerman, 1976) and was mixed in a 1:1 ratio with vaporized HCl (Granett and Taylor, 1976). Both gas and dust were diluted with air prior to being mixed. The plants were assessed for injury 24 hours after exposure.

Results and Discussion

Table 13 summarizes the results of the injury sustained by the zinnias. The data are graphed in Figure 11 with lines of best fit. Increased injury with aluminum oxide is slight and not statistically significant.

Table 13. Leaf injury on 27-day-old zinnia plants exposed to HCl gas alone or with Al_2O_3 particles.

Range HCl gas concentration[1]	Leaves injured[2]		Leaf area injured[3]	
	HCl	HCl + Al_2O_3[4]	HCl	HCl + Al_2O_3[4]
0-10	26	--	3	--
11-20	11	24	1	3
21-30	74	80	27	34
31-40	83	80	46	41

[1]Gas in mg HCl m^{-3}

[2]Percent leaves injured of those exposed

[3]Weighted average of percent leaf area injured

[4]Ratio of HCl:Al_2O_3 = 1:1 by weight

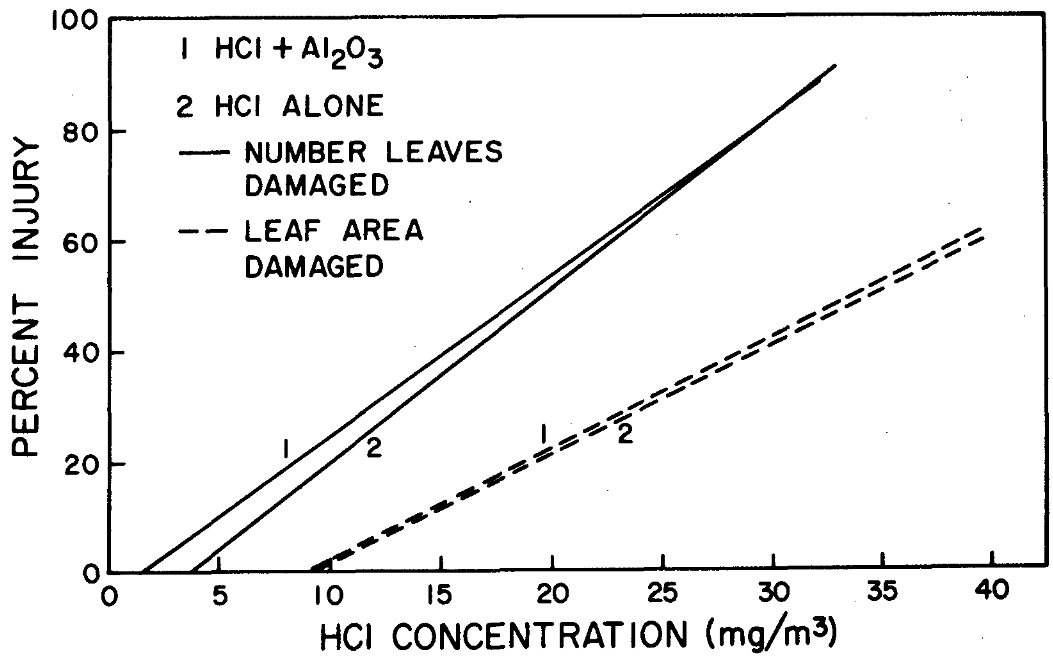

Figure 11. Injury on zinnia leaves of plants exposed to HCl or to HCl + Al_2O_3.

High density mixing of Al_2O_3 and HCl

Materials and Methods

A glass cone was constructed and placed at the output of the particulate generator (see Figure 1B). This cone has a side port allowing the vaporized HCl gas to mix directly with the Al_2O_3 before either toxicant is greatly diluted by the intake air to the chamber. French dwarf double marigolds, 50 days old, were exposed to gas and particulates mixed in this way or to the same pollutants diluted first and then mixed at the air intake manifold. Controls were exposed to HCl gas alone. Exposures were each 10 minutes long and the HCl gas concentrations ranged from 4 to 43 mg m^{-3}. Al_2O_3 was about equal to HCl concentration. The plants were graded for injury 24 hours after exposure.

Results and Discussion

The data have been grouped and summarized in 5 mg m^{-3} concentration increments in Table 14. The linear regression lines of best fit in Figure 12 are based on all the data. Data variability is large and valid comparisons cannot be made. No significant relationship could be seen between injury and relative humidity (33-47%), temperature (24-35 C), light intensity (1.3-4.8 x 10^4 ergs cm^{-2} sec^{-1}), or time of day (0830-1400 hr). Differences between injury and concentration for the three treatments are slight and re-emphasize the negligible effect of Al_2O_3. The study also indicates that damage by Al_2O_3 and HCl was not based on the type of mixing, at least under these conditions.

Table 14. Leaf injury on marigold plants exposed to 10 minutes of HCl gas with or without Al_2O_3 particles.

Range HCl gas concentration ($mg\ m^{-3}$)	HCl alone		High density mixing of HCl and Al_2O_3		HCl injected separately from Al_2O_3	
	% leaves[1]	% area[2]	% leaves[1]	% area[2]	% leaves[1]	% area[2]
0-10	0	0	0	0	-	-
11-20	0	0	0	0	0	0
21-30	18	4	21	5	24	8
31-40	38	12	35	13	42	12
41-50	39	3	2	1	20	2

[1]Percent leaves injured of those exposed

[2]Percent area damaged is a weighted average of leaf damage

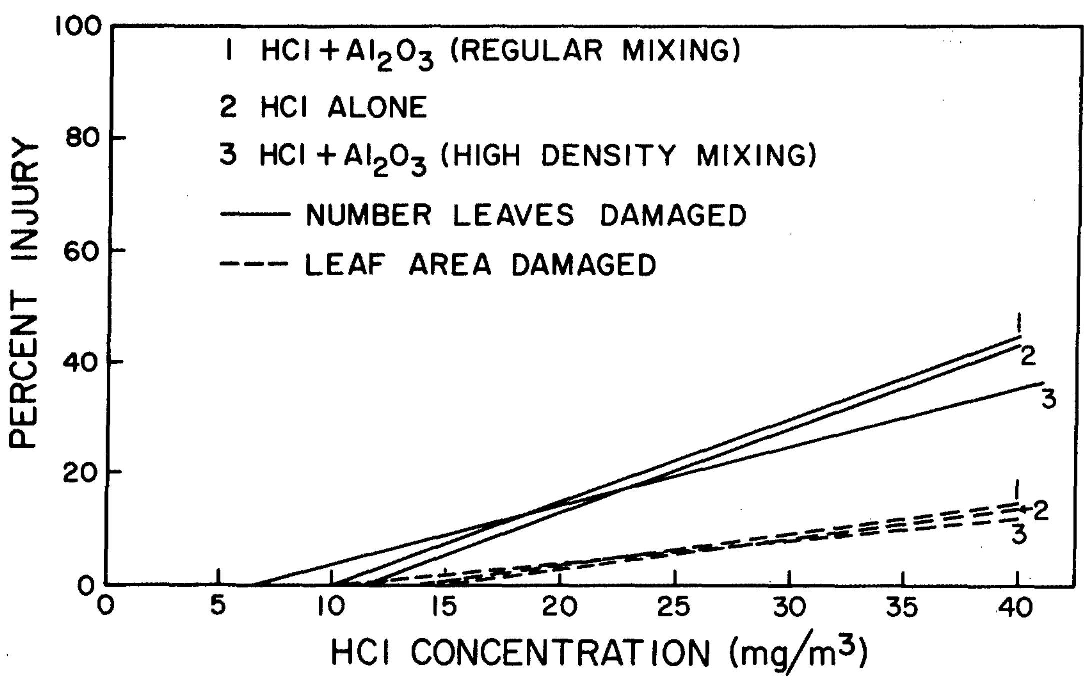

Figure 12. Effect of mixing HCl and Al_2O_3 before (high density) or after (regular) dilution with carrier air.

Cumulative effect of HCl and Al_2O_3 on marigolds

Materials and Methods

In another study Senator Dirksen marigolds were exposed on three successive weeks, starting at 5 weeks of age, to high or low concentrations of HCl gas alone or with Al_2O_3 particulates. The concentrations of the two pollutants were approximately equal and ranged from 5 to 25 mg m^{-3}. Temperature and relative humidity ranges were 32-37 C and 57-64%, respectively. Injury was assessed 24 hours after each fumigation. Two weeks after final exposure, the plants were harvested, dried at 70 C, and weighed. Sixteen plants were fumigated during each exposure.

Results and Discussion

Table 15 summarizes the injury and final dry weights for the marigold plants for the two experiments. A multiple range test performed on the dry weights showed small but significant differences between high concentrations of HCl gas and controls whether the Al_2O_3 dust was present or not. Thus, there appears to be a long term effect from HCl, but the dust does not contribute significantly to injury at any concentration.

Constant humidity during Al_2O_3 exposures

Materials and Methods

Relative humidity during exposure is thought to affect the amount of injury an exposed plant sustains. In this experiment, the relative humidity was held at 50% (49.4 ± 5.4%) by mixing steam with the intake air. A 400 watt halide lamp provided a constant background illumination, useful on the cloudy days experienced during these exposures. Senator Dirksen marigolds, 30 days old, were exposed for 15 minutes to concentrations of HCl gas, Al_2O_3 particles, or an equal mixture of the two pollutants. Five plants were exposed for each treatment and the whole series was repeated three times. The injury on the marigolds was recorded 24 hours post-exposure.

Results and Discussion

Table 16 summarizes the data of the 36 exposures. Plants had less injury after exposure to both pollutants compared to only HCl, but this was not statistically significant. There was no injury from Al_2O_3 alone.

The data were analyzed on the basis of each of the three pollutant treatments using an SPSS (Nie et al., 1975) multiple-regression program. Injury could be correlated with concentration of HCl and HCl plus Al_2O_3 but not with Al_2O_3 alone where there was no injury. Table 17 lists the r^2 correlation term as each factor is added to the analysis. Relative humidity accounted for the second highest r^2 values. The r^2 values for temperature and light, whose effects were interrelated, were almost

Table 15. Leaf injury and dry weights of marigolds exposed weekly to high or low concentrations or HCl gas with or without Al_2O_3 particles.

Pollutant		Leaves injured[3] Week Number			Dry Weights[4]		
HCl[1]	Al_2O_3[2]	1	2	3	Top	Root	Total
Experiment 1							
0	-	0	0	0	6.27 A	1.33 A	7.60 A
11	-	34	19	11	6.14 AB	1.37 AB	7.57 AB
13	15	45	36	17	6.38 AB	1.40 AB	7.79 AB
17	-	78	62	51	5.88 AB	1.21 AB	6.95 B
19	25	80	74	52	5.83 B	1.28 B	7.11 B
Temperature		37 C	32 C	34 C			
Relative Humidity		64%	60%	64%			
Experiment 2							
0	-	0	0	0	4.94 A	1.33 A	6.27 A
6	-	9	19	5	4.79 AB	1.23 AB	6.03 AB
6	10	32	16	8	4.71 ABC	1.28 AB	5.99 AB
13	-	80	57	80	4.34 C	1.14 B	5.48 B
13	21	70	53	71	4.46 BC	1.17 AB	5.63 B
Temperature		37 C	33 C	34 C			
Relative Humidity		64%	62%	57%			

[1]Concentration of HCl in mg m^{-3}, average of 3 fumigations

[2]Concentration of Al_2O_3 in mg m^{-3}, average of 3 fumigations

[3]Percent of leaves injured of those exposed, an average of 16 plants

[4]Average dry weight, in grams, of 16 plants after drying at 70 C
Weights followed by same letters are not significantly different at 5% level by Duncan's multiple range test

negligible. The ambient humidity study also listed in Table 16 was an earlier marigold experiment. In neither study were there significant differences between HCl and HCl plus Al_2O_3. In the controlled humidity test, however, more of the correlation (r^2) is due to the HCl and less to humidity than was the earlier case. The random variability ($1-r^2$) due to experimental

Table 16. Injury to marigold plants exposed to HCl, Al_2O_3, or HCl + Al_2O_3 at 50% relative humidity[1].

Theoretical concentration[2]	HCl			Al_2O_3			HCl + Al_2O_3		
	Conc[3]	LD[4]	LA[5]	Conc[3]	LD[4]	LA[5]	Conc[3]	LD[4]	LA[5]
10	13	0	0	10	0	0	13/10	0	0
15	15	20	6	15	0	0	16/15	9	1
25	25	70	19	25	0	0	24/25	64	19
35	33	72	22	35	0	0	33/35	70	22

[1]Relative humidity, controlled with live steam, averaged 49.4 ± 5.4%

[2]Pollutant concentation in mg m^{-3}

[3]Average pollutant concentration of three exposures in mg m^{-3}

[4]Percent of total leaves that are damaged

[5]Percent of leaf area that show injury

Table 17. Dependent variables in multiple regression analysis of two marigold studies with HCl and HCl plus Al_2O_3.

Variables	Average variable values		r^2 computed values	
	HCl	HCl + Al_2O_3	HCl	HCl + Al_2O_3
Controlled humidity test				
HCl concentration			.647	.734
Relative humidity (%)	50 ± 7	50 ± 4	.043	.072
Temperature (C)	39 ± 3	38 ± 3	.033	.009
Light intensity ($10^5 \frac{ergs}{cm^2 sec}$)	1.3 ± 1.2	1.2 ± 1.4	.013	.012
(Random)	--	--	(.275)	(.103)
Ambient humidity test				
HCl concentration			.348	.410
Relative humidity (%)	59 ± 10	58 ± 11	.218	.178
Temperature (C)	37 ± 4	36 ± 4	.009	.000
Light intensity ($10^5 \frac{ergs}{cm^2 sec}$)	2.0 ± 1.4	2.0 ± 1.6	.001	.000
(Random)	--	--	(.423)	(.411)

error, plants, or other factors decreases with controlled humidity and injury can be more directly attributable to HCl.

EFFECT OF HCl GAS ON YIELD OF PLANTS

In the cumulative HCl and Al_2O_3 experiments, it was noted that three exposures at weekly intervals produced a small but significant reduction in final plant biomass. This was true for the higher HCl exposures with Al_2O_3 having little effect. To further examine the effect of short fumigations on developing plants, radishes were studied. In two related experiments, radishes were exposed either once each week for 4 successive weeks or were exposed only one time during the five-week growing period. Plants were harvested five weeks after sowing, when roots had developed.

Effect of cumulative HCl exposures on radish yields

Materials and Methods

A population of Comet radish was fumigated at weekly intervals starting one week after sowing seeds. The gas concentration, 6 mg HCl m^{-3}, was chosen to produce only slight leaf injury. All fumigations were 20 minutes long. Of the original population of 50 plants, 10 were sacrificed just prior to the weekly fumigation and their fresh and dry weights were recorded. The last fumigation was four weeks after sowing and final harvest was one week later. Control plants were placed in the chamber and exposed to filtered air and were also sacrificed weekly. The two replicas of the experiment were fumigated separately on the same day.

Results and Discussion

The dry weight data are presented in Table 18 and Figure 13 shows relative growth rate curves. The roots of plants exposed during the first week and harvested prior to the second week produced the only data which were significantly different from the controls. With these plants the cotyledons had been seriously injured and this was reflected in the reduced growth during the week following fumigation. The retardation of root development was not carried into later weeks. That there was no final harvest reduction in biomass from the cumulative exposures may have been due to the low HCl concentration. Radish plants seemed to out-grow any sustained damage in a relatively short time.

Effect of single exposures on radish yields

Materials and Methods

In this experiment, groups of ten radish plants were exposed once, at some time in the four weeks after sowing. The exposures, 20 mg HCl m^{-3} for 20 minutes produced substantial leaf injury. The plants were all harvested

Table 18. Biomass yield of radish plants exposed to HCl gas weekly with sacrificial harvests.

Week Sacrificed	Average Root dry weights (mg) Control		Average Root dry weights (mg) Exposed	Average Total dry weights (mg) Control	Average Total dry weights (mg) Exposed
1	2		2	12	12
2	7	**	5	43	45
3	60		57	277	292
4	662		563	1179	1041
5	1076		1126	1902	1948

**Control plants were significantly higher than exposed plants at 1% level

five weeks after planting. Fresh and dry weights were recorded. Exposures were replicated on the same day.

Results and Discussion

Table 19 lists the stage at which the radishes were exposed to HCl and summarizes the root weights of all plants in the experiment. The fresh and dry weights of the roots are illustrated by the graphs in Figure 14. There was a significant reduction in the final root weight when 3-week-old plants were exposed. The two-week-old plants were also quite sensitive to permanent damage from the gas. The very young and the older, maturing plants were relatively tolerant to damage from the exposures. The statistical analysis used was a randomized complete block design with replications as blocks. There were five treatments. When the interaction between replications and treatments was significant in comparison to between-plant variability, as in the harvested root weights, the interaction mean square term was used to test treatments since the between-plant variability (residual mean square) was too small to use as an experimental error term. This analysis is more demanding than more conventional analysis.

These data are important showing that single fumigations made at the right time in the development cycle can affect harvest several weeks later. Gas concentration is important. Long-term damage was evident here where radishes underwent only a single, strong fumigation episode but not in the previous study where the plants each received several weak doses. Several HCl exposures at low concentrations can apparently be handled safely by the growing plant.

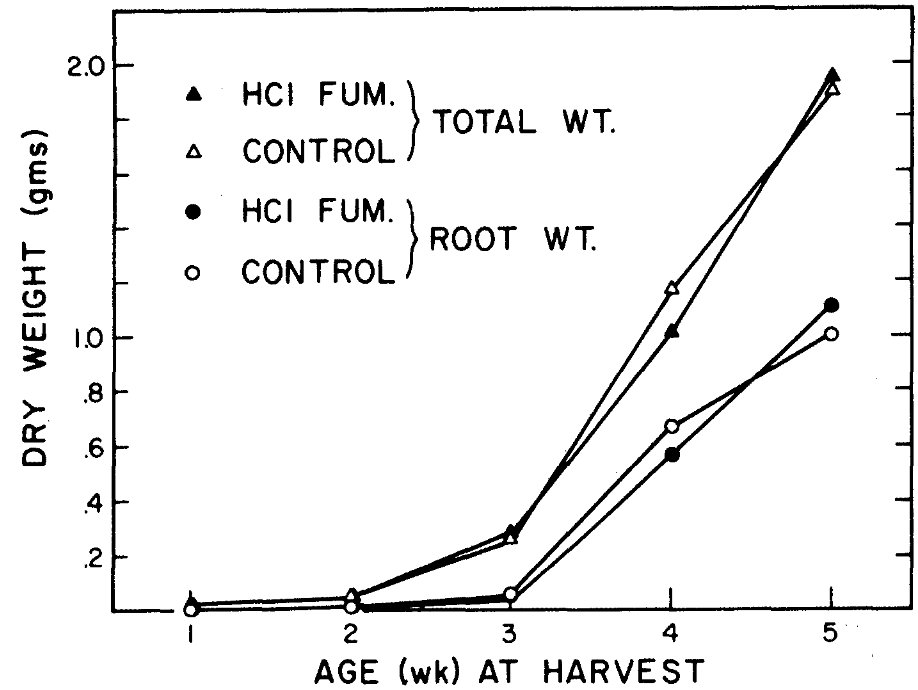

Figure 13. Effect on dry weight of weekly HCl exposures to radish plants with sacrificial harvests.

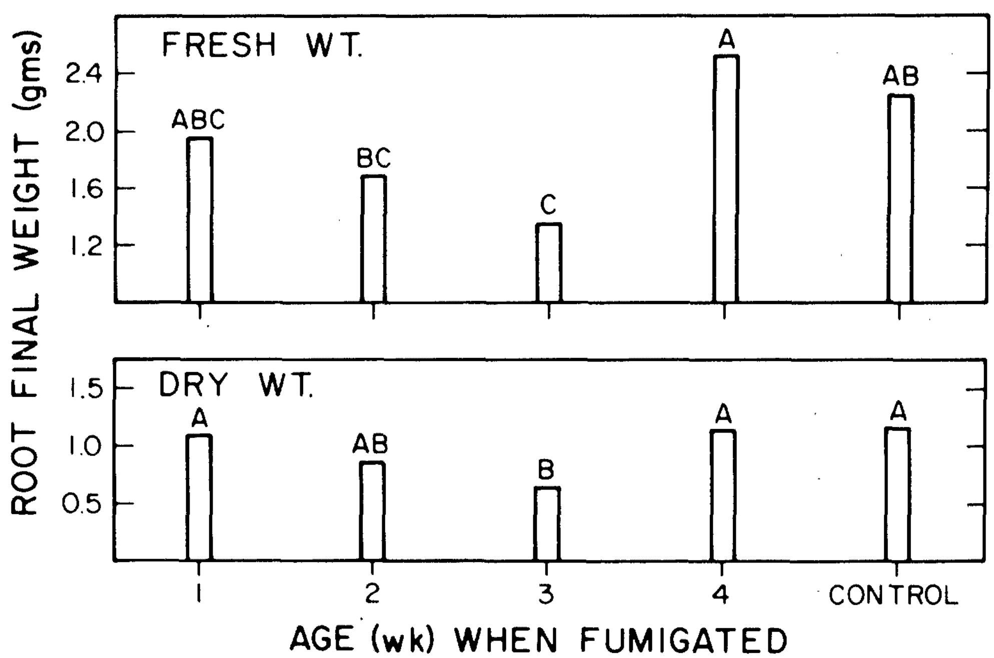

Figure 14. Effect on final root weight (yield) of radish plants exposed once during development. Letters refer to multiple range analysis of weight means. Bars with the same letter are not significantly different.

Table 19. Harvest data and analysis of roots from radishes exposed to 20 minutes of 20 mg m^{-3} HCl gas once during plant development.

Age at exposure[1]	Fresh weight: Average root weights[2]	Fresh weight: M-range analysis[3]	Dry weight: Average root weights[2]	Dry weight: M-range analysis[3]
No fumigation	22.205	AB	1.155	A
1	19.492	ABC	1.080	A
2	16.791	BC	0.850	AB
3	13.403	C	0.636	B
4	25.070	A	1.133	A

[1] Plant age in weeks after seeds were sown

[2] Average weights of 20 roots, in grams

[3] Analysis by Duncan's new multiple range test separates different means at the 5% level of significance: means followed by the same letter are not significantly different

EFFECT OF POLLUTANTS ON MYCORRHIZAE

In the last decade much attention has been focused on the ability of root-inhabiting vesicular-arbuscular (VA) mycorrhizae to improve plant growth. Uptake of phosphorus and other mineral nutrients by the plant is enhanced by this symbiotic relationship, especially in soils of low fertility (Tinker, 1975). Factors which stress plants such as pesticides, shading, pruning, and plant nutrition, have been shown to decrease mycorrhizae in the soil. Plant stress due to air pollution may also decrease mycorrhizae. In the present study, changes in plant growth and mycorrhizal population after exposure to HCl gas, HCl in combination with Al_2O_3 particulates, or to ozone, were examined.

Materials and Methods

Half of a population of one-week-old citrus and zinnia seedlings were inoculated during transplanting with the endomycorrihizal fungus *Glomus fasciculatus*. Inoculum consisted of 10 g of soil and roots of sudan grass (*Sorghum vulgare* var. *sudanense* [Staph]) containing hyphae, vesicles, arbuscles, and chlamydospores of *G. fasciculatus*. Chlamydospore concentration was approximately 145 and 190 spores/g soil for citrus and zinnia, respectively. The rest of the plant population were non-mycorrhizal controls. All seedlings were maintained in the greenhouse. They were grown in 5-inch pots containing autoclaved sandy loam and were fertilized weekly with half-strength nutrient solution deficient in phosphorus.

At 5, 12, and 16 weeks, sets of 10 mycorrhizal and 10 non-mycorrhizal citrus seedlings were exposed for 20 minutes to 110-140 mg m^{-3} HCl gas, to 110-140 mg m^{-3} HCl gas plus 39 mg m^{-3} Al_2O_3 particulates, or for four hours to 100 pphm ozone. Sets of 10 mycorrhizal and 10 non-mycorrhizal zinnia seedlings were exposed at 1, 2, 4, and 8 weeks after inoculation to 25 mg m^{-3} HCl gas for 20 minutes or to 69 pphm ozone for four hours. Symptoms were noted at the time of and 48 hours after each fumigation. Unexposed groups of mycorrhizal and non-mycorrhizal plants served as fumigation controls.

The citrus and zinnia were harvested one week after the last fumigations at 17 and 9 weeks, respectively. Each plant was removed carefully from the soil and shoot height and leaf, stem, and root dry weights were recorded. The number of chlamydospores of G. fasciculatus associated with each seedling was determined by wet sieving and decanting of a 200 cc soil aliquot through 1, 2.4, and 45 openings/mm Tyler wire mesh screens.

Results

Citrus exposed to HCl exhibited moderate marginal leaf necrosis. Approximately 20-25% of each affected leaf surface was necrotic. Ozone produced no visible symptoms on citrus at the toxicant levels used. HCl produced marginal necrosis and bleaching on zinnia. Ozone-exposed zinnia exhibited chlorosis and light flecking of the upper leaf surface. Symptoms on both citrus and zinnia were fully expressed within 48 hours post-fumigation with either HCl or ozone. Stress could sometimes be seen immediately after fumigation and was usually manifested by chlorosis or necrosis in the stressed leaves.

Mycorrhizal citrus exposed to HCl or to HCl plus Al_2O_3 were 36% and 47% taller, respectively, than corresponding non-mycorrhizal seedlings but were not significantly different than the unexposed mycorrhizal controls. Ozone-exposed mycorrhizal citrus were not significantly taller than non-mycorrhizal citrus but were 37% shorter than the non-exposed mycorrhizal controls. Chlamydospore production by G. fasciculatus in inoculated seedlings was reduced 50% in ozone-exposed plants while HCl and HCl plus Al_2O_3-exposed seedlings were not statistically different from the unexposed controls. Table 20 summarizes both height and spore data. Weight data for the citrus are seen in Table 21. Unexposed mycorrhizal controls had significantly heavier dry weights than HCl- or ozone-exposed mycorrhizal seedlings. However, the total dry weights of pollutant-treated mycorrhizal and non-mycorrhizal seedlings were not significantly different.

The data for zinnia seedlings are in Tables 22 and 23. Mycorrhizal zinnia plants, either unfumigated or fumigated with HCl or ozone, were significantly taller and heavier than their non-mycorrhizal counterparts. There were no statistical differences, however, between pollutants given the same mycorrhizal treatments. No significant trends were noted in the spore counts.

Table 20. Effect of several pollutants on height and mycorrhizal spore production of mycorrhizal and non-mycorrhizal citrus seedlings.

	Height[1]		Spores[3]	
Treatment	+Mycorrhizae	-Mycorrhizae	+Mycorrhizae	-Mycorrhizae
$HCl + Al_2O_3$	27.8 A[2]	14.6 E	907 A[4]	0
HCl	23.6 ABC	15.1 DE	905 A	0
Ozone	19.7 CDE	18.3 CDE	528 B	0
Control	31.2 A	22.6 BCD	1067 A	0

[1]Height data is mean of 10 plants, in cm

[2]Height means of all 8 treatment combinations followed by the same letter are not significantly different at 5% level by Duncan's multiple range test

[3]Number of G. fasiculatus chlamydospores found in 200 cc of soil, average of soil from 10 plants

[4]Only data from mycorrhizal plants were analyzed by the multiple-range test

Discussion

This study indicates that the endomycorrhizal symbiotic association on citrus can be influenced by ozone but is not influenced by HCl gas or the combination of HCl and Al_2O_3. Plant size and nutrition are closely related to mycorrhizal infection and spore production and can, in some cases, be utilized to monitor the extent of the symbiotic relationship. The observation of reduced plant height and dry weight in the ozone treatments correlates well with the observed spore reduction in the same plants. An effect of ozone on mycorrhizae which operates indirectly through the plant is hypothesized, although the factors which operate to produce such an effect are not known. HCl, in contrast, does not seem to alter the host/fungus relationship in citrus and, due to better host nutrition when the fungus is present, may actually lessen the severity of the effects of HCl on plant growth.

The mycorrhizal association with zinnia does not seem to be affected by either HCl or ozone. This suggests that observed effects on mycorrhizae due to air pollutants may vary significantly from host to host and could depend on the host response to pollutant stress.

Table 21. Effect of several pollutants on total dry weight of mycorrhizal and non-mycorrhizal citrus.

Treatment	Total dry weight in grams[1] +Mycorrhizae	-Mycorrhizae
$HCl + Al_2O_3$	3.862 AB	2.191 D
HCl	2.878 BCD	2.162 D
Ozone	2.551 D	2.596 CD
Control	4.232 A	3.725 ABC

[1]Mean of 10 plants. All treatment combinations were analyzed by Duncan's multiple range test and data followed by the same letter are not significantly different at 5% level

Table 22. Effect of HCl and ozone on height of mycorrhizal and non-mycorrhizal zinnia.

	Height in cm[1]			
	Replica 1		Replica 2	
Treatment	+Mycorrhizae	-Mycorrhizae	+Mycorrhizae	-Mycorrhizae
HCl	19.1 A	8.4 B	13.1 BC	11.6 BC
Ozone	17.2 A	8.0 B	22.5 A	7.9 C
Control	16.4 A	9.9 B	14.5 B	10.2 C

[1]Mean of 10 plants. Data in the same replica followed by the same letter were not significantly different at 5% level by Duncan's multiple range test

Table 23. Effect of HCl and ozone on stem and leaf dry weight of mycorrhizal and non-mycorrhizal zinnia.

	Dry weights in mg[1]			
	Stem		Leaf	
Treatment	+Mycorrhizae	-Mycorrhizae	+Mycorrhizae	-Mycorrhizae
HCl	105 A	38 B	76 A	44 B
Ozone	128 A	44 B	83 A	42 B
Control	114 A	45 B	74 A	40 B

[1]Mean of 20 plants. Stem and leaf data were analyzed separately by Duncan's multiple range test. No significant differences exist between data followed by the same letter.

VISUAL INJURY RESPONSE ON SELECTED PLANTS

An objective of this project is to predict the amount of plant injury that will occur after exposure to HCl gas. One method to achieve such prediction models is to determine that threshold concentration at which visual plant injury occurs. Lerman (1976; Lerman et al., 1976) fumigated particular plant species at various ages, times of the year, and at different doses (exposure x HCl concentration) as plants were available. The data were analyzed with a multiple regression computer program and the results were complex equations which took plant age as well as gas concentration and length of exposure into account.

In succeeding studies, age was kept constant and the exposure time and concentrations were varied to define a threshold concentration below which no plants were damaged. The empirical nature of the experiments necessitated many exposures involving hundreds of plants. Instead of determining multiple regression lines, we were interested in fitting the data, usually expressed as number of plants or number of leaves damaged, to a hyperbolic curve relating damage to concentration. Random experimental errors and large variability led to difficulties in fitting our data to expected curves (Granett and Taylor, 1976).

More recently, our experiments have been designed to minimize variability (age, growth conditions, etc.) whenever possible. The resulting data have been analyzed more critically.

Materials and Methods

Plants were fumigated at a certain age or stage of development. Usually the plants were quite young, often less than 7 weeks from sowing. An exposure matrix was determined by fumigating a few plants. The matrix usually consisted of three exposure periods 5, 10, and 20 minutes, and up to five HCl concentrations, each double the previous one (e.g. 10, 20, 40, and 80 mg m^{-3}). All exposures in the matrix were performed during the same day or on successive days. Temperature, relative humidity, light intensity, time of day and actual HCl concentrations were recorded. In most cases the plants were checked for stress soon after the exposure was complete and were then moved to greenhouse benches. At 24 to 48 hours post-exposure, the leaves were graded for visible injury using a 0-4 system which took severity and area damage into account. Bifacial necrosis and/or abaxial glazing were the most common damage. After grading, plant damage was summarized as + or - injury, number of leaves injured of total exposed, and estimated percent of area injured. Similarly, a single fumigation (all plants exposed at the same time) was summarized with percent plants injured, percent leaves injured, and percent of leaf area damaged, in addition to the necessary background data (time, concentration, temperature, date, etc.). The summary was transferred to IBM cards for computer availability. Basic programs were revised to accept our data and to compute the desired analysis. Final graphs were plotted when analysis was complete. We are using only leaf numbers (percent leaf injury) for all

the work reported here.

Results and Discussion

Instead of deriving that concentration point at which damage becomes visible, it was felt useful to construct lines which could predict approximate concentrations for some damage level. An ED_{50} (estimated damage) could then be calculated to predict the gas concentration necessary to produce any injury on 50% of the leaves exposed. If the data clustered around the low damage end of the lines, the line was extrapolated to 50% to find ED_{50}.

Linear regression calculations required transformation of percent damage to arcsin values[1] for more normal distribution (Little and Hills, 1972) and a $\log_{10}$ scale was used for gas concentrations. Regression lines, seen in Figures 15 and 16, were plotted when a product-moment correlation coefficient (r) was significant at the 5% level or better. The correlation coefficients, along with the corresponding coefficients of determination (r^2) are tabulated by species in Table 24. The coefficient of determination is useful to indicate which portion of the variation in the dependent variable (% damage) is explained by the independent variable ($\log_{10}$ HCl concentration). As r^2 approaches 1.0, more of the damage can be related to the concentration and less is due to other variables such as temperature, humidity, or other causes of experimental error (Nie et al., 1975; Sokal and Rohlf, 1969). Twenty exposure levels of 11 plant species produced data which satisfied the criteria and were significant for linear regression analysis. The number of points in Table 24 refer to the different exposure combinations each plant received for a given time. For some exposure times tested, there were just not enough fumigations at different concentrations to produce significant linear regression even though the r and r^2 values were high.

Probit analysis (Finney, 1971) of the injury data was performed when the linear regression analysis produced significant regression sum of squares and the data fulfilled certain other criteria (Finney, 1971; Fisher and Yates, 1963). Table 25 lists those experiments in which probit analysis could be performed and the estimated concentrations predicted to damage to some degree 50% of the leaves exposed (ED_{50}). Probit computations were performed by a program written by M.J. Garber (UCR Statistic Department). Percent leaves damaged was used with natural mortality set equal to zero, and HCl concentration were on a $\log_{10}$ scale. Figures 17 and 18 contain the probit regression lines and Table 26 compares probit analysis with linear and multiple regression values.

Presenting threshold data as an ED_{50} is more accurate for prediction than finding that single point when damage is first seen on any one plant in a fumigation. To further investigate the minimal damage, the slopes and intercepts of the linear regression lines were used to calculate ED_{10} values, that gas concentration at which 10% injury could be expected on particular plants after particular exposure durations. These values are tabulated in

[1]Injury = arcsin $(\text{percent damage})^{\frac{1}{2}}$

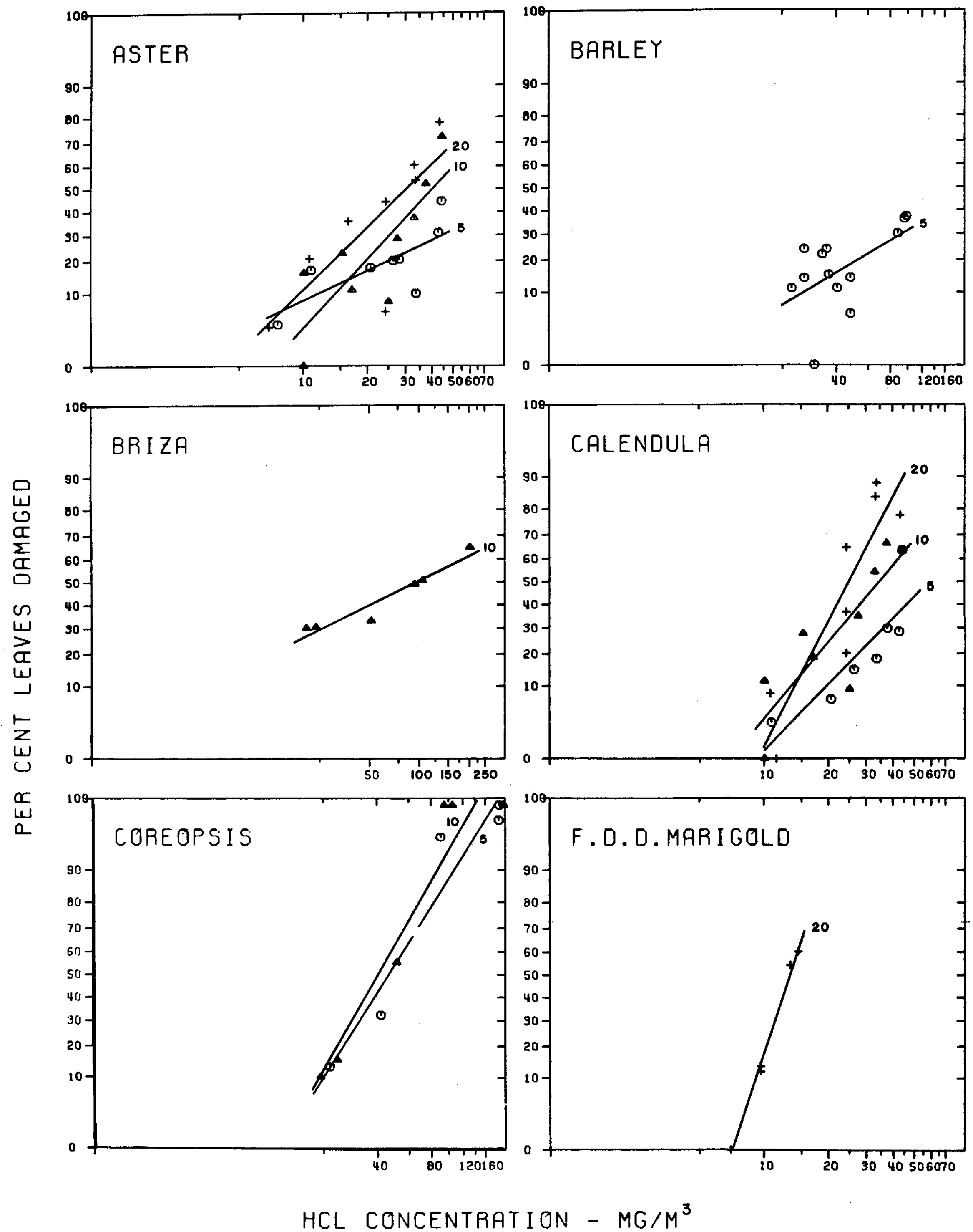

Figure 15. Linear regression analysis of six plant species. Leaf damage is in percent injury of the total leaves exposed on an arcsin scale. Concentration is on a $\log_{10}$ scale. Exposure durations were 5 (O), 10 (▲), and 20 (+) minutes.

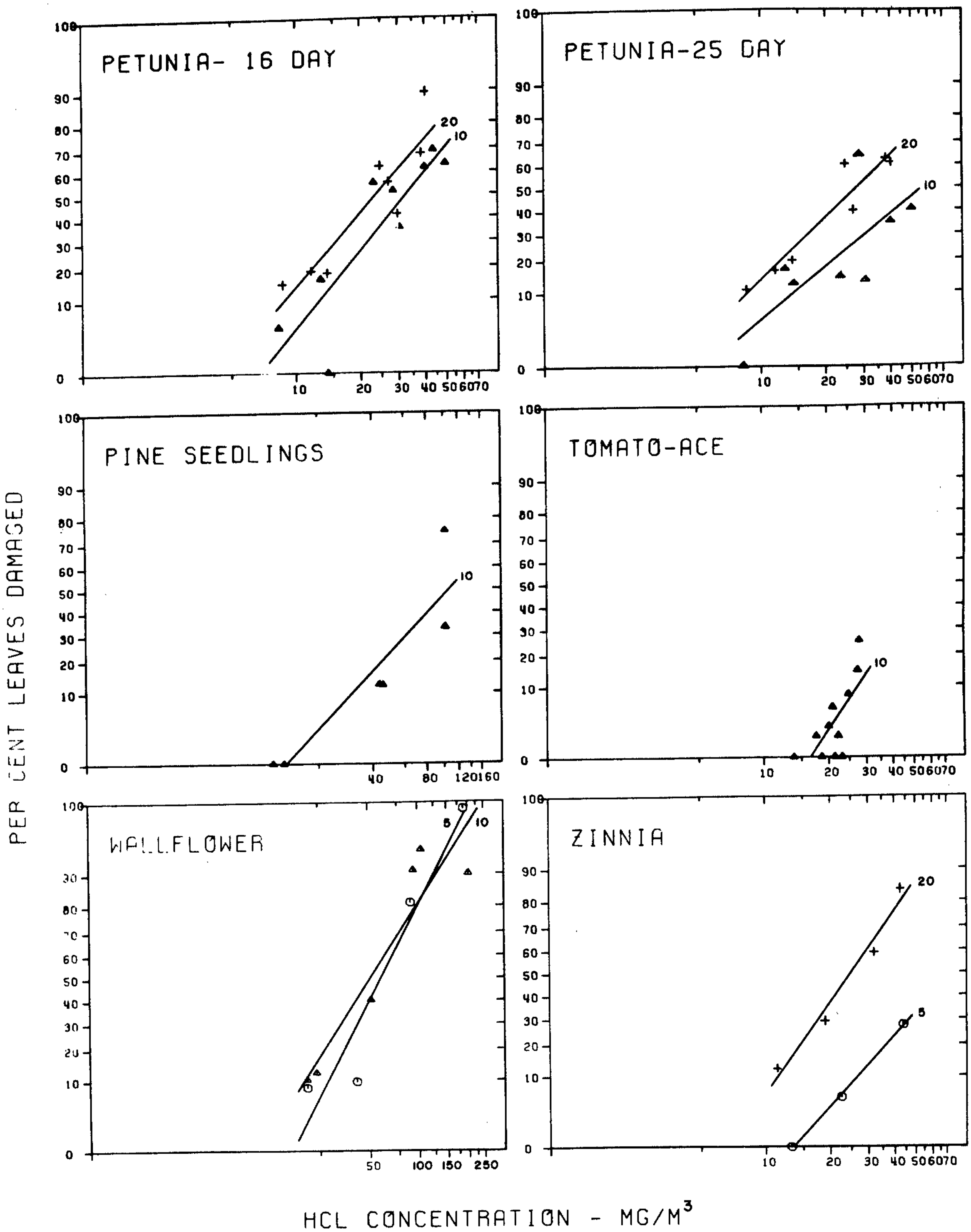

Figure 16. Linear regression analysis of five plant species. Leaf damage is on an arcsin scale and concentration is on a $\log_{10}$ scale. Exposure durations were 5 (0), 10 (▲), and 20 (+) minutes.

Table 24. Linear regression analysis of the leaf injury on particular species after exposure to HCl gas.

Species	Age (days)	Exposure duration (minutes)	Number of points	ED_{50} (mg m^{-3})	r	r^2	Significance * - 5% level ** - 10% level NS - No signif.
Aster	31	5	8	128	.751	.564	*
	46	10	9	41	.817	.667	**
	54	20	8	30	.795	.630	*
Barley		5	13	254	.572	.327	*
Bean	13	5	4	23	.854	.729	NS
	13	10	4	16	.842	.709	NS
Briza	49	10	6	97	.954	.920	**
Calendula	46	5	7	59	.850	.722	*
	54	10	9	35	.859	.730	**
	54	20	8	25	.898	.806	NS
Coreopsis	50	5	5	46	.963	.928	**
	50	10	6	41	.955	.912	**
Marigold		5	5	31	.868	.754	NS
		20	5	13	.996	.992	**
Petunia	16	10	9	33	.876	.768	**
	16	20	9	24	.917	.840	**
Petunia	25	10	8	57	.759	.661	*
	25	20	7	28	.951	.905	**
Pine	42	10	6	107	.917	.840	**
Tomato	34	10	11	58	.719	.516	*
Wallflower	51	5	4	59	.958	.918	*
	51	10	6	51	.936	.877	**
Zinnia	8	5	3	30	.981	.962	NS
	8	10	3	24	.979	.958	NS
	8	20	3	16	.900	.811	NS
Zinnia	16	5	3	74	.999	.998	*
	16	10	3	37	.948	.898	NS
	16	20	3	24	.878	.768	NS
Zinnia	27	10	3	33	.957	.916	NS
	27	20	4	25	.986	.973	*

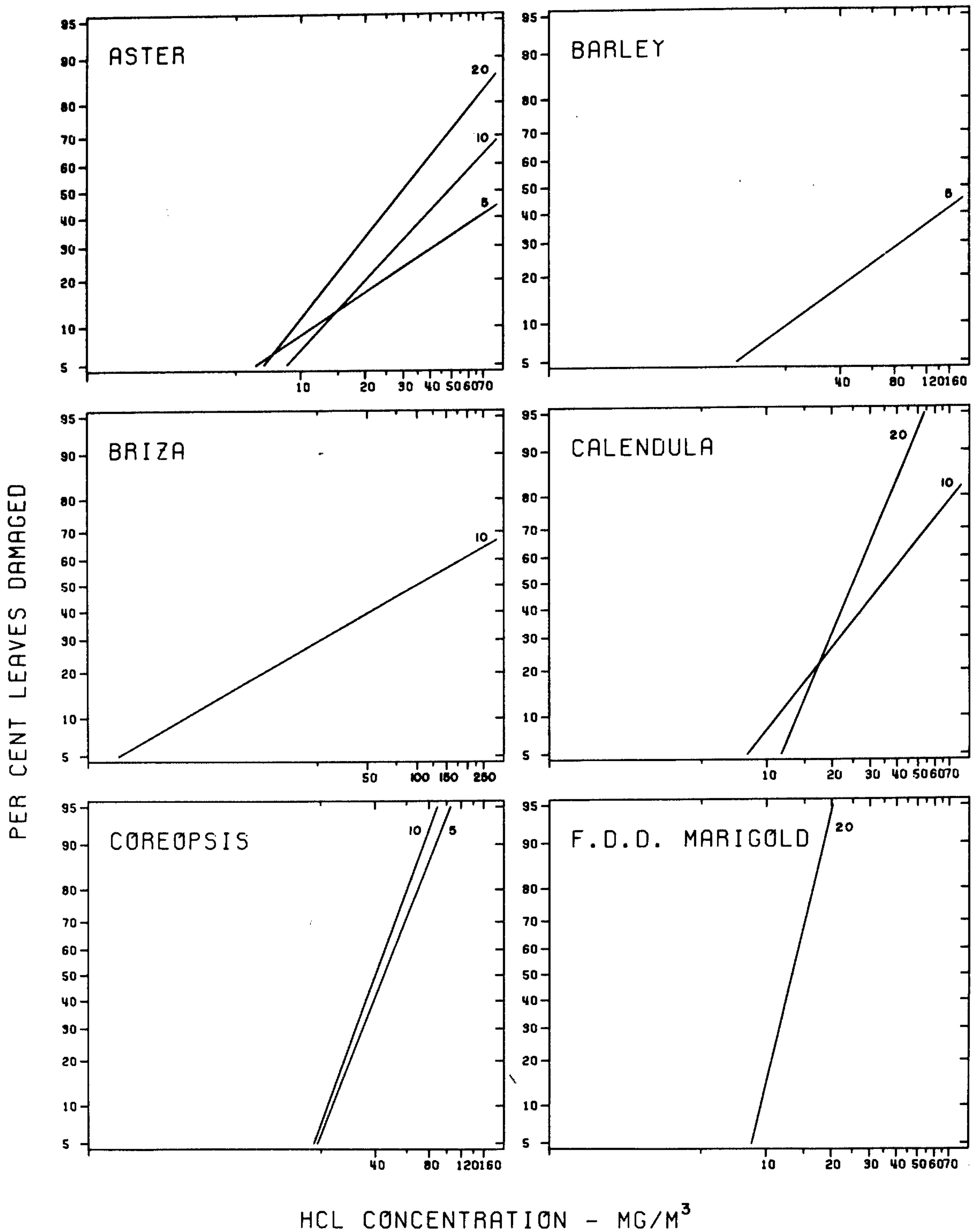

Figure 17. Probit analysis of six plant species. Probit scale is the probability of certain percent of the total leaves exposed will be injured at given concentration ($\log_{10}$ scale) after a 5, 10, or 20 minute exposure.

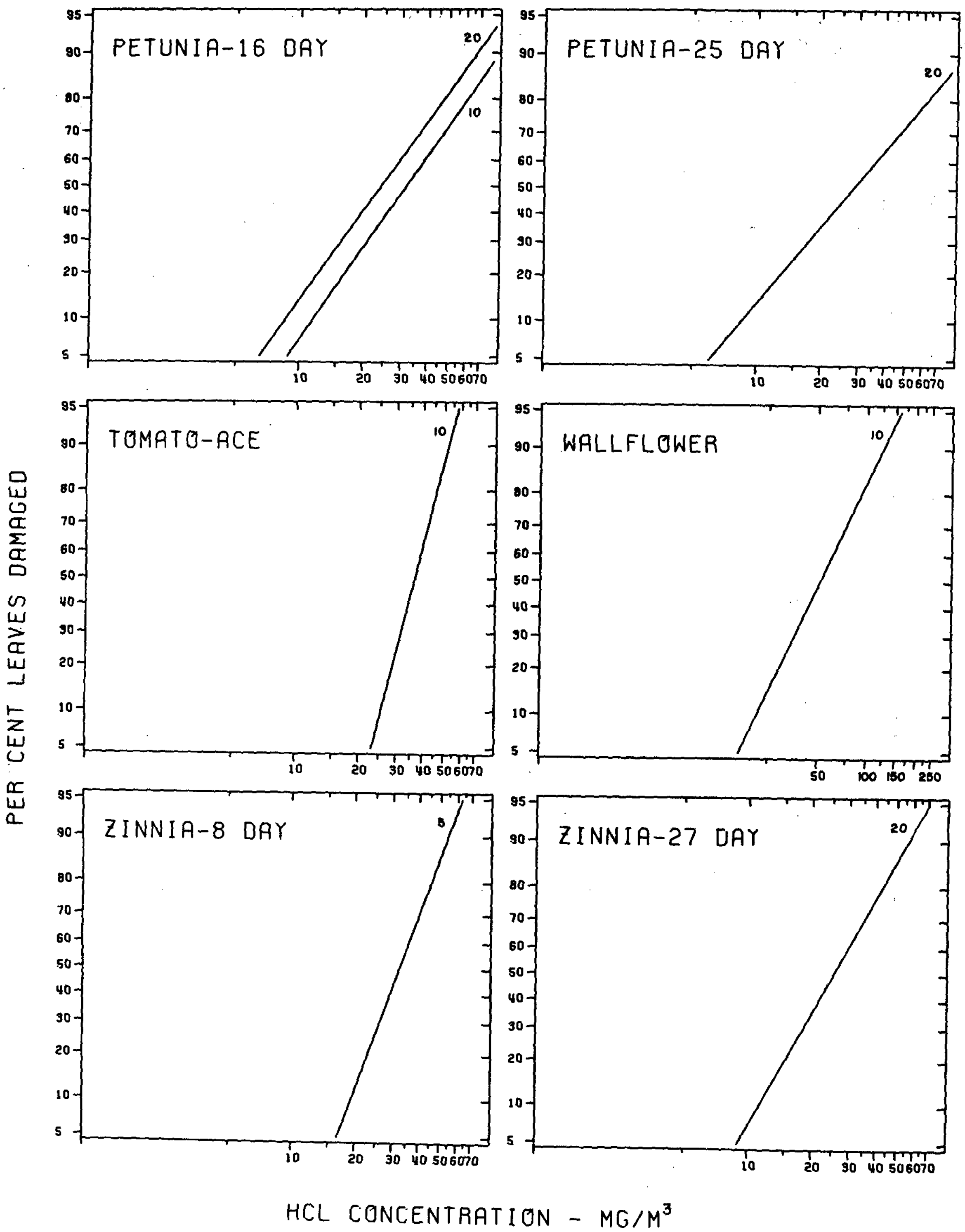

Figure 18. Probit analysis of four plant species. Probit scale is the probability that a certain percent of the total leaves exposed will be injured at a given concentration ($\log_{10}$ scale) after a 5, 10, or 20 minute exposure.

Table 25. Probit analysis of the percent leaves of various plant species injured by exposure to HCl gas.

Species	Exposure duration (minutes	Probit analysis values[1] (mg HCl m^{-1}) ED_{50}	Confidence limits Upper	Lower
Aster	5	102	49	hi
	10	49	31	1500
	20	30	19	118
Barley	5	239	109	hi
Briza	10	98	82	117
Calendula	10	35	25	126
	20	25	16	35
Coreopsis	5	45	5	218
	10	40	31	51
Marigold	20	13	13	14
Petunia (16 day)	10	32	28	38
	20	24	21	28
(25 day)	20	28	24	36
Tomato	10	37	31	79
Wallflower	10	51	29	84
Zinnia	20	25	23	28

[1]Computer calculations of probit analysis determine the ED_{50} and the upper and lower 5% confidence limits for that value. The ED_{50} is the concentration in mg HCl m^{-3} necessary to cause injury on 50% of the leaves exposed.

Table 27. The values are mathematically derived so some variation from observed can be expected. To estimate the more tolerant and sensitive plants, the highest and lowest ED values were selected, resulting in Table 28. It is noted that petunia and marigolds are more sensitive while pine is more tolerant to damage by HCl gas. Distinctions in other plants are more difficult.

There were fewer experimental variables in these studies compared to earlier efforts as the plants were grown at the same time and fumigations were within a day, if not hours, of each other, instead of weeks or months apart. This method provided more statistically useful data with fewer plants. The results are a further attempt to define, in the simplest terms possible, a complex biological system containing many known and unknown variables.

Table 26. Comparisons between calculations of ED_{50} of leaf injury from HCl gas on selected plant species.

Species	Exposure duration (minutes)	ED_{50} Multiple Regression[2]	Linear Regression	Probit
Aster	5	-	128	102
	10	-	41	49
	20	26	30	30
Barley	5	-	254	239
Briza	10	-	97	98
Calendula	10	-	59	35
	20	13	35	25
Coreopsis	5	-	46	45
	10	-	41	40
Marigold	20	9	13	13
Petunia (16 day)	10	-	33	32
	20	-	24	24
(25 day)	20	-	28	28
Tomato	10	-	58	37
Wallflower	10	-	51	51
Zinnia (16 day)	5	-	74	32
(27 day)	20	13	25	25

[1] ED_{50} is the HCl conentration in mg m^{-3} necessary to produce injury on 50% of the leaves exposed

[2] Calculated from equations from Lerman et al., 1976

Table 27. HCl concentrations calculated for expected damage of 10% and 50% of the leaves of a population of plants.

Species	Exposure duration (minutes)	ED_{10}	ED_{50}
Aster	5	12[1]	128
	10	14	41
	20	10	30
Barley	5	26	254
Briza	10	5	97
Calendula	5	20	59
	10	13	35
Coreopsis	5	20	46
	10	19	41
Marigold	20	9	13
Petunia (16 day)	10	13	33
	20	9	24
Petunia (25 day)	10	14	57
	20	9	28
Pine	10	31	107
Tomato	10	28	58
Wallflower	5	28	59
	10	20	51
Zinnia (16 day)	5	27	74
Zinnia (27 day)	20	12	25

[1]Concentration for each ED is in mg HCl m^{-3}

Table 28. Linear regression estimates of species tolerant or sensitive to HCl gas.

Tolerant plants		Sensitive plants	
ED_{10}	ED_{50}	ED_{10}	ED_{50}
Zinnia	Aster	Briza	Marigold
Tomato	Pine	Marigold	Petunia
Wallflower	Barley	Petunia	Zinnia
Pine		Aster	

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